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(54) Title: MOLECULES FOR DIAGNOSTICS AND THERAPEUTICS

(57) Abstract: The present invention provides purified human polynucleotides for diagnostics and therapeutics (dithp). Also en-
compassed are the polypeptides (DITHP) encoded by dithp. The invention also provides for the use of dithp, or complements,
oligonucleotides, or fragments thereof in diagnostic assays. The invention further provides for vectors and host cells containing
dithp for the expression of DITHP. The invention additionally provides for the use of isolated and purified DITHP to induce antibod-
ies and to screen libraries of compounds and the use of anti-DITHP antibodies in diagnostic assays. Also provided are microarrays
containing dithp and methods of use.

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WO 01/62927 A2

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MOLECULES FOR DIAGNOSTICS AND THERAPEUTICS

TECHNICAL FIELD

The present invention relates to human molecules and to the use of these sequences in the diagnosis, study, prevention, and treatment of diseases associated with, as well as effects of exogenous compounds on, the expression of human molecules.

BACKGROUND OF THE INVENTION

The human genome is comprised of thousands of genes, many encoding gene products that function in the maintenance and growth of the various cells and tissues in the body. Aberrant expression or mutations in these genes and their products is the cause of, or is associated with, a variety of human diseases such as cancer and other cell proliferative disorders, autoimmune/inflammatory disorders, infections, developmental disorders, endocrine disorders, metabolic disorders, neurological disorders, gastrointestinal disorders, transport disorders, and connective tissue disorders. The identification of these genes and their products is the basis of an ever-expanding effort to find markers for early detection of diseases, and targets for their prevention and treatment. Therefore, these genes and their products are useful as diagnostics and therapeutics. These genes may encode, for example, enzyme molecules, molecules associated with growth and development, biochemical pathway molecules, extracellular information transmission molecules, receptor molecules, intracellular signaling molecules, membrane transport molecules, protein modification and maintenance molecules, nucleic acid synthesis and modification molecules, adhesion molecules, antigen recognition molecules, secreted and extracellular matrix molecules, cytoskeletal molecules, ribosomal molecules, electron transfer associated molecules, transcription factor molecules, chromatin molecules, cell membrane molecules, and organelle associated molecules.

For example, cancer represents a type of cell proliferative disorder that affects nearly every tissue in the body. A wide variety of molecules, either aberrantly expressed or mutated, can be the cause of, or involved with, various cancers because tissue growth involves complex and ordered patterns of cell proliferation, cell differentiation, and apoptosis. Cell proliferation must be regulated to maintain both the number of cells and their spatial organization. This regulation depends upon the appropriate expression of proteins which control cell cycle progression in response to extracellular signals such as growth factors and other mitogens, and intracellular cues such as DNA damage or nutrient starvation. Molecules which directly or indirectly modulate cell cycle progression fall into several categories, including growth factors and their receptors, second messenger and signal transduction proteins, oncogene products, tumor-suppressor proteins, and mitosis-promoting factors.

Aberrant expression or mutations in any of these gene products can result in cell proliferative disorders such as cancer. Oncogenes are genes generally derived from normal genes that, through abnormal expression or mutation, can effect the transformation of a normal cell to a malignant one (oncogenesis). Oncoproteins, encoded by oncogenes, can affect cell proliferation in a variety of ways and include
5 growth factors, growth factor receptors, intracellular signal transducers, nuclear transcription factors, and cell-cycle control proteins. In contrast, tumor-suppressor genes are involved in inhibiting cell proliferation. Mutations which cause reduced function or loss of function in tumor-suppressor genes result in aberrant cell proliferation and cancer. Although many different genes and their products have been found to be associated with cell proliferative disorders such as cancer, many more may exist that
10 are yet to be discovered.

DNA-based arrays can provide a simple way to explore the expression of a single polymorphic gene or a large number of genes. When the expression of a single gene is explored, DNA-based arrays are employed to detect the expression of specific gene variants. For example, a p53 tumor suppressor gene array is used to determine whether individuals are carrying mutations that predispose them to
15 cancer. A cytochrome p450 gene array is useful to determine whether individuals have one of a number of specific mutations that could result in increased drug metabolism, drug resistance or drug toxicity.

DNA-based array technology is especially relevant for the rapid screening of expression of a large number of genes. There is a growing awareness that gene expression is affected in a global fashion. A genetic predisposition, disease or therapeutic treatment may affect, directly or indirectly, the
20 expression of a large number of genes. In some cases the interactions may be expected, such as when the genes are part of the same signaling pathway. In other cases, such as when the genes participate in separate signaling pathways, the interactions may be totally unexpected. Therefore, DNA-based arrays can be used to investigate how genetic predisposition, disease, or therapeutic treatment affects the expression of a large number of genes.

Enzyme Molecules

The cellular processes of biogenesis and biodegradation involve a number of key enzyme classes including oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. These enzyme classes are each comprised of numerous substrate-specific enzymes having precise and well
30 regulated functions. These enzymes function by facilitating metabolic processes such as glycolysis, the tricarboxylic cycle, and fatty acid metabolism; synthesis or degradation of amino acids, steroids, phospholipids, alcohols, etc.; regulation of cell signalling, proliferation, inflammation, apoptosis, etc., and through catalyzing critical steps in DNA replication and repair, and the process of translation.

Oxidoreductases

Many pathways of biogenesis and biodegradation require oxidoreductase (dehydrogenase or reductase) activity, coupled to the reduction or oxidation of a donor or acceptor cofactor. Potential cofactors include cytochromes, oxygen, disulfide, iron-sulfur proteins, flavin adenine dinucleotide (FAD), and the nicotinamide adenine dinucleotides NAD and NADP (Newsholme, E.A. and A.R. Leech (1983) Biochemistry for the Medical Sciences, John Wiley and Sons, Chichester, U.K., pp. 779-793). Reductase activity catalyzes the transfer of electrons between substrate(s) and cofactor(s) with concurrent oxidation of the cofactor. The reverse dehydrogenase reaction catalyzes the reduction of a cofactor and consequent oxidation of the substrate. Oxidoreductase enzymes are a broad superfamily of proteins that catalyze numerous reactions in all cells of organisms ranging from bacteria to plants to humans. These reactions include metabolism of sugar, certain detoxification reactions in the liver, and the synthesis or degradation of fatty acids, amino acids, glucocorticoids, estrogens, androgens, and prostaglandins. Different family members are named according to the direction in which their reactions are typically catalyzed; thus they may be referred to as oxidoreductases, oxidases, reductases, or dehydrogenases. In addition, family members often have distinct cellular localizations, including the cytosol, the plasma membrane, mitochondrial inner or outer membrane, and peroxisomes.

Short-chain alcohol dehydrogenases (SCADs) are a family of dehydrogenases that only share 15% to 30% sequence identity, with similarity predominantly in the coenzyme binding domain and the substrate binding domain. In addition to the well-known role in detoxification of ethanol, SCADs are also involved in synthesis and degradation of fatty acids, steroids, and some prostaglandins, and are therefore implicated in a variety of disorders such as lipid storage disease, myopathy, SCAD deficiency, and certain genetic disorders. For example, retinol dehydrogenase is a SCAD-family member (Simon, A. et al. (1995) *J. Biol. Chem.* 270:1107-1112) that converts retinol to retinal, the precursor of retinoic acid. Retinoic acid, a regulator of differentiation and apoptosis, has been shown to down-regulate genes involved in cell proliferation and inflammation (Chai, X. et al. (1995) *J. Biol. Chem.* 270:3900-3904). In addition, retinol dehydrogenase has been linked to hereditary eye diseases such as autosomal recessive childhood-onset severe retinal dystrophy (Simon, A. et al. (1996) *Genomics* 36:424-430).

Propagation of nerve impulses, modulation of cell proliferation and differentiation, induction of the immune response, and tissue homeostasis involve neurotransmitter metabolism (Weiss, B. (1991) *Neurotoxicology* 12:379-386; Collins, S.M. et al. (1992) *Ann. N.Y. Acad. Sci.* 664:415-424; Brown, J.K. and H. Imam (1991) *J. Inherit. Metab. Dis.* 14:436-458). Many pathways of neurotransmitter metabolism require oxidoreductase activity, coupled to reduction or oxidation of a cofactor, such as NAD⁺/NADH (Newsholme, E.A. and A.R. Leech (1983) Biochemistry for the

Medical Sciences, John Wiley and Sons, Chichester, U.K. pp. 779-793). Degradation of catecholamines (epinephrine or norepinephrine) requires alcohol dehydrogenase (in the brain) or aldehyde dehydrogenase (in peripheral tissue). NAD⁺-dependent aldehyde dehydrogenase oxidizes 5-hydroxyindole-3-acetate (the product of 5-hydroxytryptamine (serotonin) metabolism) in the brain, blood platelets, liver and pulmonary endothelium (Newsholme, supra, p. 786). Other neurotransmitter degradation pathways that utilize NAD⁺/NADH-dependent oxidoreductase activity include those of L-DOPA (precursor of dopamine, a neuronal excitatory compound), glycine (an inhibitory neurotransmitter in the brain and spinal cord), histamine (liberated from mast cells during the inflammatory response), and taurine (an inhibitory neurotransmitter of the brain stem, spinal cord and retina) (Newsholme, supra, pp. 790, 792). Epigenetic or genetic defects in neurotransmitter metabolic pathways can result in a spectrum of disease states in different tissues including Parkinson disease and inherited myoclonus (McCance, K.L. and S.E. Huether (1994) Pathophysiology, Mosby-Year Book, Inc., St. Louis MO, pp. 402-404; Gundlach, A.L. (1990) FASEB J. 4:2761-2766).

Tetrahydrofolate is a derivatized glutamate molecule that acts as a carrier, providing activated one-carbon units to a wide variety of biosynthetic reactions, including synthesis of purines, pyrimidines, and the amino acid methionine. Tetrahydrofolate is generated by the activity of a holoenzyme complex called tetrahydrofolate synthase, which includes three enzyme activities: tetrahydrofolate dehydrogenase, tetrahydrofolate cyclohydrolase, and tetrahydrofolate synthetase. Thus, tetrahydrofolate dehydrogenase plays an important role in generating building blocks for nucleic and amino acids, crucial to proliferating cells.

3-Hydroxyacyl-CoA dehydrogenase (3HACD) is involved in fatty acid metabolism. It catalyzes the reduction of 3-hydroxyacyl-CoA to 3-oxoacyl-CoA, with concomitant oxidation of NAD to NADH, in the mitochondria and peroxisomes of eukaryotic cells. In peroxisomes, 3HACD and enoyl-CoA hydratase form an enzyme complex called bifunctional enzyme, defects in which are associated with peroxisomal bifunctional enzyme deficiency. This interruption in fatty acid metabolism produces accumulation of very-long chain fatty acids, disrupting development of the brain, bone, and adrenal glands. Infants born with this deficiency typically die within 6 months (Watkins, P. et al. (1989) J. Clin. Invest. 83:771-777; Online Mendelian Inheritance in Man (OMIM), #261515). The neurodegeneration that is characteristic of Alzheimer's disease involves development of extracellular plaques in certain brain regions. A major protein component of these plaques is the peptide amyloid- β (A β), which is one of several cleavage products of amyloid precursor protein (APP). 3HACD has been shown to bind the A β peptide, and is overexpressed in neurons affected in Alzheimer's disease. In addition, an antibody against 3HACD can block the toxic effects of A β in a cell culture model of Alzheimer's disease (Yan, S. et al. (1997) Nature 389:689-695; OMIM,

#602057).

Steroids, such as estrogen, testosterone, corticosterone, and others, are generated from a common precursor, cholesterol, and are interconverted into one another. A wide variety of enzymes act upon cholesterol, including a number of dehydrogenases. Steroid dehydrogenases, such as the hydroxysteroid dehydrogenases, are involved in hypertension, fertility, and cancer (Duax, W.L. and D. Ghosh (1997) *Steroids* 62:95-100). One such dehydrogenase is 3-oxo-5- α -steroid dehydrogenase (OASD), a microsomal membrane protein highly expressed in prostate and other androgen-responsive tissues. OASD catalyzes the conversion of testosterone into dihydrotestosterone, which is the most potent androgen. Dihydrotestosterone is essential for the formation of the male phenotype during embryogenesis, as well as for proper androgen-mediated growth of tissues such as the prostate and male genitalia. A defect in OASD that prevents the conversion of testosterone into dihydrotestosterone leads to a rare form of male pseudohermaphroditis, characterized by defective formation of the external genitalia (Andersson, S. et al. (1991) *Nature* 354:159-161; Labrie, F. et al. (1992) *Endocrinology* 131:1571-1573; OMIM #264600). Thus, OASD plays a central role in sexual differentiation and androgen physiology.

17 β -hydroxysteroid dehydrogenase (17 β HSD6) plays an important role in the regulation of the male reproductive hormone, dihydrotestosterone (DHTT). 17 β HSD6 acts to reduce levels of DHTT by oxidizing a precursor of DHTT, 3 α -diol, to androsterone which is readily glucuronidated and removed from tissues. 17 β HSD6 is active with both androgen and estrogen substrates when expressed in embryonic kidney 293 cells. At least five other isozymes of 17 β HSD have been identified that catalyze oxidation and/or reduction reactions in various tissues with preferences for different steroid substrates (Biswas, M.G. and D.W. Russell (1997) *J. Biol. Chem.* 272:15959-15966). For example, 17 β HSD1 preferentially reduces estradiol and is abundant in the ovary and placenta. 17 β HSD2 catalyzes oxidation of androgens and is present in the endometrium and placenta. 17 β HSD3 is exclusively a reductive enzyme in the testis (Geissler, W.M. et al. (1994) *Nat. Genet.* 7:34-39). An excess of androgens such as DHTT can contribute to certain disease states such as benign prostatic hyperplasia and prostate cancer.

Oxidoreductases are components of the fatty acid metabolism pathways in mitochondria and peroxisomes. The main beta-oxidation pathway degrades both saturated and unsaturated fatty acids, while the auxiliary pathway performs additional steps required for the degradation of unsaturated fatty acids. The auxiliary beta-oxidation enzyme 2,4-dienoyl-CoA reductase catalyzes the removal of even-numbered double bonds from unsaturated fatty acids prior to their entry into the main beta-oxidation pathway. The enzyme may also remove odd-numbered double bonds from unsaturated fatty acids (Koivuranta, K.T. et al. (1994) *Biochem. J.* 304:787-792; Smeland, T.E. et al. (1992) *Proc.*

Natl. Acad. Sci. USA 89:6673-6677). 2,4-dienoyl-CoA reductase is located in both mitochondria and peroxisomes. Inherited deficiencies in mitochondrial and peroxisomal beta-oxidation enzymes are associated with severe diseases, some of which manifest themselves soon after birth and lead to death within a few years. Defects in beta-oxidation are associated with Reye's syndrome, Zellweger
5 syndrome, neonatal adrenoleukodystrophy, infantile Refsum's disease, acyl-CoA oxidase deficiency, and bifunctional protein deficiency (Suzuki, Y. et al. (1994) Am. J. Hum. Genet. 54:36-43; Hoefler, supra; Cotran, R.S. et al. (1994) Robbins Pathologic Basis of Disease, W.B. Saunders Co., Philadelphia PA, p.866). Peroxisomal beta-oxidation is impaired in cancerous tissue. Although neoplastic human breast epithelial cells have the same number of peroxisomes as do normal cells,
10 fatty acyl-CoA oxidase activity is lower than in control tissue (el Bouhtoury, F. et al. (1992) J. Pathol. 166:27-35). Human colon carcinomas have fewer peroxisomes than normal colon tissue and have lower fatty-acyl-CoA oxidase and bifunctional enzyme (including enoyl-CoA hydratase) activities than normal tissue (Cable, S. et al. (1992) Virchows Arch. B Cell Pathol. Incl. Mol. Pathol. 62:221-226). Another important oxidoreductase is isocitrate dehydrogenase, which catalyzes the conversion
15 of isocitrate to α -ketoglutarate, a substrate of the citric acid cycle. Isocitrate dehydrogenase can be either NAD or NADP dependent, and is found in the cytosol, mitochondria, and peroxisomes. Activity of isocitrate dehydrogenase is regulated developmentally, and by hormones, neurotransmitters, and growth factors.

Hydroxypyruvate reductase (HPR), a peroxisomal 2-hydroxyacid dehydrogenase in the
20 glycolate pathway, catalyzes the conversion of hydroxypyruvate to glycerate with the oxidation of both NADH and NADPH. The reverse dehydrogenase reaction reduces NAD^+ and NADP^+ . HPR recycles nucleotides and bases back into pathways leading to the synthesis of ATP and GTP. ATP and GTP are used to produce DNA and RNA and to control various aspects of signal transduction and energy metabolism. Inhibitors of purine nucleotide biosynthesis have long been employed as
25 antiproliferative agents to treat cancer and viral diseases. HPR also regulates biochemical synthesis of serine and cellular serine levels available for protein synthesis.

The mitochondrial electron transport (or respiratory) chain is a series of oxidoreductase-type enzyme complexes in the mitochondrial membrane that is responsible for the transport of electrons from NADH through a series of redox centers within these complexes to oxygen, and the coupling of
30 this oxidation to the synthesis of ATP (oxidative phosphorylation). ATP then provides the primary source of energy for driving a cell's many energy-requiring reactions. The key complexes in the respiratory chain are NADH:ubiquinone oxidoreductase (complex I), succinate:ubiquinone oxidoreductase (complex II), cytochrome c_1 -b oxidoreductase (complex III), cytochrome c oxidase (complex IV), and ATP synthase (complex V) (Alberts, B. et al. (1994) Molecular Biology of the

Cell, Garland Publishing, Inc., New York NY, pp. 677-678). All of these complexes are located on the inner matrix side of the mitochondrial membrane except complex II, which is on the cytosolic side. Complex II transports electrons generated in the citric acid cycle to the respiratory chain. The electrons generated by oxidation of succinate to fumarate in the citric acid cycle are transferred
5 through electron carriers in complex II to membrane bound ubiquinone (Q). Transcriptional regulation of these nuclear-encoded genes appears to be the predominant means for controlling the biogenesis of respiratory enzymes. Defects and altered expression of enzymes in the respiratory chain are associated with a variety of disease conditions.

Other dehydrogenase activities using NAD as a cofactor are also important in mitochondrial
10 function. 3-hydroxyisobutyrate dehydrogenase (3HBD), important in valine catabolism, catalyzes the NAD-dependent oxidation of 3-hydroxyisobutyrate to methylmalonate semialdehyde within mitochondria. Elevated levels of 3-hydroxyisobutyrate have been reported in a number of disease states, including ketoacidosis, methylmalonic acidemia, and other disorders associated with deficiencies in methylmalonate semialdehyde dehydrogenase (Rougraff, P.M. et al. (1989) J. Biol.
15 Chem. 264:5899-5903).

Another mitochondrial dehydrogenase important in amino acid metabolism is the enzyme isovaleryl-CoA-dehydrogenase (IVD). IVD is involved in leucine metabolism and catalyzes the oxidation of isovaleryl-CoA to 3-methylcrotonyl-CoA. Human IVD is a tetrameric flavoprotein that is encoded in the nucleus and synthesized in the cytosol as a 45 kDa precursor with a mitochondrial
20 import signal sequence. A genetic deficiency, caused by a mutation in the gene encoding IVD, results in the condition known as isovaleric acidemia. This mutation results in inefficient mitochondrial import and processing of the IVD precursor (Vockley, J. et al. (1992) J. Biol. Chem. 267:2494-2501).

Transferases

Transferases are enzymes that catalyze the transfer of molecular groups. The reaction may
25 involve an oxidation, reduction, or cleavage of covalent bonds, and is often specific to a substrate or to particular sites on a type of substrate. Transferases participate in reactions essential to such functions as synthesis and degradation of cell components, regulation of cell functions including cell signaling, cell proliferation, inflammation, apoptosis, secretion and excretion. Transferases are involved in key steps in disease processes involving these functions. Transferases are frequently
30 classified according to the type of group transferred. For example, methyl transferases transfer one-carbon methyl groups, amino transferases transfer nitrogenous amino groups, and similarly denominated enzymes transfer aldehyde or ketone, acyl, glycosyl, alkyl or aryl, isoprenyl, saccharyl, phosphorous-containing, sulfur-containing, or selenium-containing groups, as well as small enzymatic groups such as Coenzyme A.

Acyl transferases include peroxisomal carnitine octanoyl transferase, which is involved in the fatty acid beta-oxidation pathway, and mitochondrial carnitine palmitoyl transferases, involved in fatty acid metabolism and transport. Choline O-acetyl transferase catalyzes the biosynthesis of the neurotransmitter acetylcholine.

5 Amino transferases play key roles in protein synthesis and degradation, and they contribute to other processes as well. For example, the amino transferase 5-aminolevulinic acid synthase catalyzes the addition of succinyl-CoA to glycine, the first step in heme biosynthesis. Other amino transferases participate in pathways important for neurological function and metabolism. For example, glutamine-phenylpyruvate amino transferase, also known as glutamine transaminase K (GTK), catalyzes several
10 reactions with a pyridoxal phosphate cofactor. GTK catalyzes the reversible conversion of L-glutamine and phenylpyruvate to 2-oxoglutarate and L-phenylalanine. Other amino acid substrates for GTK include L-methionine, L-histidine, and L-tyrosine. GTK also catalyzes the conversion of kynurenine to kynurenic acid, a tryptophan metabolite that is an antagonist of the N-methyl-D-aspartate (NMDA) receptor in the brain and may exert a neuromodulatory function. Alteration of the
15 kynurenine metabolic pathway may be associated with several neurological disorders. GTK also plays a role in the metabolism of halogenated xenobiotics conjugated to glutathione, leading to nephrotoxicity in rats and neurotoxicity in humans. GTK is expressed in kidney, liver, and brain. Both human and rat GTKs contain a putative pyridoxal phosphate binding site (ExPASy ENZYME: EC 2.6.1.64; Perry, S.J. et al. (1993) Mol. Pharmacol. 43:660-665; Perry, S. et al. (1995) FEBS Lett. 360:277-280; and Alberati-Giani, D. et al. (1995) J. Neurochem. 64:1448-1455). A second amino
20 transferase associated with this pathway is kynurenine/ α -aminoadipate amino transferase (AadAT). AadAT catalyzes the reversible conversion of α -aminoadipate and α -ketoglutarate to α -ketoadipate and L-glutamate during lysine metabolism. AadAT also catalyzes the transamination of kynurenine to kynurenic acid. A cytosolic AadAT is expressed in rat kidney, liver, and brain (Nakatani, Y. et al.
25 (1970) Biochim. Biophys. Acta 198:219-228; Buchli, R. et al. (1995) J. Biol. Chem. 270:29330-29335).

Glycosyl transferases include the mammalian UDP-glucouronosyl transferases, a family of membrane-bound microsomal enzymes catalyzing the transfer of glucouronic acid to lipophilic substrates in reactions that play important roles in detoxification and excretion of drugs, carcinogens,
30 and other foreign substances. Another mammalian glycosyl transferase, mammalian UDP-galactose-ceramide galactosyl transferase, catalyzes the transfer of galactose to ceramide in the synthesis of galactocerebrosides in myelin membranes of the nervous system. The UDP-glycosyl transferases share a conserved signature domain of about 50 amino acid residues (PROSITE: PDOC00359, <http://expasy.hcuge.ch/sprot/prosite.html>).

Methyl transferases are involved in a variety of pharmacologically important processes. Nicotinamide N-methyl transferase catalyzes the N-methylation of nicotinamides and other pyridines, an important step in the cellular handling of drugs and other foreign compounds. Phenylethanolamine N-methyl transferase catalyzes the conversion of noradrenalin to adrenalin. 6-O-methylguanine-DNA methyl transferase reverses DNA methylation, an important step in
5 carcinogenesis. Uroporphyrin-III C-methyl transferase, which catalyzes the transfer of two methyl groups from S-adenosyl-L-methionine to uroporphyrinogen III, is the first specific enzyme in the biosynthesis of cobalamin, a dietary enzyme whose uptake is deficient in pernicious anemia. Protein-arginine methyl transferases catalyze the posttranslational methylation of arginine residues in
10 proteins, resulting in the mono- and dimethylation of arginine on the guanidino group. Substrates include histones, myelin basic protein, and heterogeneous nuclear ribonucleoproteins involved in mRNA processing, splicing, and transport. Protein-arginine methyl transferase interacts with proteins upregulated by mitogens, with proteins involved in chronic lymphocytic leukemia, and with interferon, suggesting an important role for methylation in cytokine receptor signaling (Lin, W.-J. et al. (1996) J. Biol. Chem. 271:15034-15044; Abramovich, C. et al. (1997) EMBO J. 16:260-266; and
15 Scott, H.S. et al. (1998) Genomics 48:330-340).

Phosphotransferases catalyze the transfer of high-energy phosphate groups and are important in energy-requiring and -releasing reactions. The metabolic enzyme creatine kinase catalyzes the reversible phosphate transfer between creatine/creatine phosphate and ATP/ADP. Glycocyamine
20 kinase catalyzes phosphate transfer from ATP to guanidoacetate, and arginine kinase catalyzes phosphate transfer from ATP to arginine. A cysteine-containing active site is conserved in this family (PROSITE: PDOC00103).

Prenyl transferases are heterodimers, consisting of an alpha and a beta subunit, that catalyze the transfer of an isoprenyl group. An example of a prenyl transferase is the mammalian protein
25 farnesyl transferase. The alpha subunit of farnesyl transferase consists of 5 repeats of 34 amino acids each, with each repeat containing an invariant tryptophan (PROSITE: PDOC00703).

Saccharyl transferases are glycosylating enzymes involved in a variety of metabolic processes. Oligosaccharyl transferase-48, for example, is a receptor for advanced glycation endproducts. Accumulation of these endproducts is observed in vascular complications of diabetes, macrovascular
30 disease, renal insufficiency, and Alzheimer's disease (Thornalley, P.J. (1998) Cell Mol. Biol. (Noisy-Le-Grand) 44:1013-1023).

Coenzyme A (CoA) transferase catalyzes the transfer of CoA between two carboxylic acids. Succinyl CoA:3-oxoacid CoA transferase, for example, transfers CoA from succinyl-CoA to a recipient such as acetoacetate. Acetoacetate is essential to the metabolism of ketone bodies, which

accumulate in tissues affected by metabolic disorders such as diabetes (PROSITE: PDOC00980).

Hydrolases

Hydrolysis is the breaking of a covalent bond in a substrate by introduction of a molecule of water. The reaction involves a nucleophilic attack by the water molecule's oxygen atom on a target
5 bond in the substrate. The water molecule is split across the target bond, breaking the bond and generating two product molecules. Hydrolases participate in reactions essential to such functions as synthesis and degradation of cell components, and for regulation of cell functions including cell signaling, cell proliferation, inflammation, apoptosis, secretion and excretion. Hydrolases are involved in key steps in disease processes involving these functions. Hydrolytic enzymes, or hydrolases, may
10 be grouped by substrate specificity into classes including phosphatases, peptidases, lysophospholipases, phosphodiesterases, glycosidases, and glyoxalases.

Phosphatases hydrolytically remove phosphate groups from proteins, an energy-providing step that regulates many cellular processes, including intracellular signaling pathways that in turn control cell growth and differentiation, cell-cell contact, the cell cycle, and oncogenesis.

15 Lysophospholipases (LPLs) regulate intracellular lipids by catalyzing the hydrolysis of ester bonds to remove an acyl group, a key step in lipid degradation. Small LPL isoforms, approximately 15-30 kD, function as hydrolases; larger isoforms function both as hydrolases and transacylases. A particular substrate for LPLs, lysophosphatidylcholine, causes lysis of cell membranes. LPL activity is regulated by signaling molecules important in numerous pathways, including the inflammatory
20 response.

Peptidases, also called proteases, cleave peptide bonds that form the backbone of peptide or protein chains. Proteolytic processing is essential to cell growth, differentiation, remodeling, and homeostasis as well as inflammation and immune response. Since typical protein half-lives range from hours to a few days, peptidases are continually cleaving precursor proteins to their active form,
25 removing signal sequences from targeted proteins, and degrading aged or defective proteins. Peptidases function in bacterial, parasitic, and viral invasion and replication within a host. Examples of peptidases include trypsin and chymotrypsin (components of the complement cascade and the blood-clotting cascade) lysosomal cathepsins, calpains, pepsin, renin, and chymosin (Beynon, R.J. and J.S. Bond (1994) Proteolytic Enzymes: A Practical Approach, Oxford University Press, New
30 York NY, pp. 1-5).

The phosphodiesterases catalyze the hydrolysis of one of the two ester bonds in a phosphodiester compound. Phosphodiesterases are therefore crucial to a variety of cellular processes. Phosphodiesterases include DNA and RNA endo- and exo-nucleases, which are essential to cell growth and replication as well as protein synthesis. Another phosphodiesterase is acid

sphingomyelinase, which hydrolyzes the membrane phospholipid sphingomyelin to ceramide and phosphorylcholine. Phosphorylcholine is used in the synthesis of phosphatidylcholine, which is involved in numerous intracellular signaling pathways. Ceramide is an essential precursor for the generation of gangliosides, membrane lipids found in high concentration in neural tissue. Defective
5 acid sphingomyelinase phosphodiesterase leads to a build-up of sphingomyelin molecules in lysosomes, resulting in Niemann-Pick disease.

Glycosidases catalyze the cleavage of hemiacetyl bonds of glycosides, which are compounds that contain one or more sugar. Mammalian lactase-phlorizin hydrolase, for example, is an intestinal enzyme that splits lactose. Mammalian beta-galactosidase removes the terminal galactose from
10 gangliosides, glycoproteins, and glycosaminoglycans, and deficiency of this enzyme is associated with a gangliosidosis known as Morquio disease type B. Vertebrate lysosomal alpha-glucosidase, which hydrolyzes glycogen, maltose, and isomaltose, and vertebrate intestinal sucrase-isomaltase, which hydrolyzes sucrose, maltose, and isomaltose, are widely distributed members of this family with highly conserved sequences at their active sites.

15 The glyoxylase system is involved in gluconeogenesis, the production of glucose from storage compounds in the body. It consists of glyoxylase I, which catalyzes the formation of S-D-lactoylglutathione from methylglyoxal, a side product of triose-phosphate energy metabolism, and glyoxylase II, which hydrolyzes S-D-lactoylglutathione to D-lactic acid and reduced glutathione. Glyoxylases are involved in hyperglycemia, non-insulin-dependent diabetes mellitus, the
20 detoxification of bacterial toxins, and in the control of cell proliferation and microtubule assembly.

Lyases

Lyases are a class of enzymes that catalyze the cleavage of C-C, C-O, C-N, C-S, C-(halide), P-O or other bonds without hydrolysis or oxidation to form two molecules, at least one of which contains a double bond (Stryer, L. (1995) Biochemistry W.H. Freeman and Co. New York, NY
25 p.620). Lyases are critical components of cellular biochemistry with roles in metabolic energy production including fatty acid metabolism, as well as other diverse enzymatic processes. Further classification of lyases reflects the type of bond cleaved as well as the nature of the cleaved group.

The group of C-C lyases include carboxyl-lyases (decarboxylases), aldehyde-lyases (aldolases), oxo-acid-lyases and others. The C-O lyase group includes hydro-lyases, lyases acting on
30 polysaccharides and other lyases. The C-N lyase group includes ammonia-lyases, amidine-lyases, amine-lyases (deaminases) and other lyases.

Proper regulation of lyases is critical to normal physiology. For example, mutation induced deficiencies in the uroporphyrinogen decarboxylase can lead to photosensitive cutaneous lesions in the genetically-linked disorder familial porphyria cutanea tarda (Mendez, M. et al. (1998) Am. J.

Genet. 63:1363-1375). It has also been shown that adenosine deaminase (ADA) deficiency stems from genetic mutations in the ADA gene, resulting in the disorder severe combined immunodeficiency disease (SCID) (Hershfield, M.S. (1998) Semin. Hematol. 35:291-298).

Isomerases

5 Isomerases are a class of enzymes that catalyze geometric or structural changes within a molecule to form a single product. This class includes racemases and epimerases, cis-trans-isomerases, intramolecular oxidoreductases, intramolecular transferases (mutases) and intramolecular lyases. Isomerases are critical components of cellular biochemistry with roles in metabolic energy production including glycolysis, as well as other diverse enzymatic processes (Stryer, L. (1995) Biochemistry, W.H. Freeman and Co., New York NY, pp.483-507).

Racemases are a subset of isomerases that catalyze inversion of a molecule's configuration around the asymmetric carbon atom in a substrate having a single center of asymmetry, thereby interconverting two racemers. Epimerases are another subset of isomerases that catalyze inversion of configuration around an asymmetric carbon atom in a substrate with more than one center of
15 symmetry, thereby interconverting two epimers. Racemases and epimerases can act on amino acids and derivatives, hydroxy acids and derivatives, as well as carbohydrates and derivatives. The interconversion of UDP-galactose and UDP-glucose is catalyzed by UDP-galactose-4'-epimerase. Proper regulation and function of this epimerase is essential to the synthesis of glycoproteins and glycolipids. Elevated blood galactose levels have been correlated with UDP-galactose-4'-epimerase
20 deficiency in screening programs of infants (Gitzelmann, R. (1972) Helv. Paediat. Acta 27:125-130).

Oxidoreductases can be isomerases as well. Oxidoreductases catalyze the reversible transfer of electrons from a substrate that becomes oxidized to a substrate that becomes reduced. This class of enzymes includes dehydrogenases, hydroxylases, oxidases, oxygenases, peroxidases, and reductases. Proper maintenance of oxidoreductase levels is physiologically important. For example, genetically-
25 linked deficiencies in lipoamide dehydrogenase can result in lactic acidosis (Robinson, B.H. et al. (1977) Pediat. Res. 11:1198-1202).

Another subgroup of isomerases are the transferases (or mutases). Transferases transfer a chemical group from one compound (the donor) to another compound (the acceptor). The types of groups transferred by these enzymes include acyl groups, amino groups, phosphate groups
30 (phosphotransferases or phosphomutases), and others. The transferase carnitine palmitoyltransferase is an important component of fatty acid metabolism. Genetically-linked deficiencies in this transferase can lead to myopathy (Scriver, C.R. et al. (1995) The Metabolic and Molecular Basis of Inherited Disease, McGraw-Hill, New York NY, pp.1501-1533).

Yet another subgroup of isomerases are the topoisomerases. Topoisomerases are enzymes

that affect the topological state of DNA. For example, defects in topoisomerases or their regulation can affect normal physiology. Reduced levels of topoisomerase II have been correlated with some of the DNA processing defects associated with the disorder ataxia-telangiectasia (Singh, S.P. et al. (1988) Nucleic Acids Res. 16:3919-3929).

5 Ligases

Ligases catalyze the formation of a bond between two substrate molecules. The process involves the hydrolysis of a pyrophosphate bond in ATP or a similar energy donor. Ligases are classified based on the nature of the type of bond they form, which can include carbon-oxygen, carbon-sulfur, carbon-nitrogen, carbon-carbon and phosphoric ester bonds.

10 Ligases forming carbon-oxygen bonds include the aminoacyl-transfer RNA (tRNA) synthetases which are important RNA-associated enzymes with roles in translation. Protein biosynthesis depends on each amino acid forming a linkage with the appropriate tRNA. The aminoacyl-tRNA synthetases are responsible for the activation and correct attachment of an amino acid with its cognate tRNA. The 20 aminoacyl-tRNA synthetase enzymes can be divided into two
15 structural classes, and each class is characterized by a distinctive topology of the catalytic domain. Class I enzymes contain a catalytic domain based on the nucleotide-binding Rossman fold. Class II enzymes contain a central catalytic domain, which consists of a seven-stranded antiparallel β -sheet motif, as well as N- and C- terminal regulatory domains. Class II enzymes are separated into two groups based on the heterodimeric or homodimeric structure of the enzyme; the latter group is further
20 subdivided by the structure of the N- and C-terminal regulatory domains (Hartlein, M. and S. Cusack (1995) J. Mol. Evol. 40:519-530). Autoantibodies against aminoacyl-tRNAs are generated by patients with dermatomyositis and polymyositis, and correlate strongly with complicating interstitial lung disease (ILD). These antibodies appear to be generated in response to viral infection, and coxsackie virus has been used to induce experimental viral myositis in animals.

25 Ligases forming carbon-sulfur bonds (Acid-thiol ligases) mediate a large number of cellular biosynthetic intermediary metabolism processes involve intermolecular transfer of carbon atom-containing substrates (carbon substrates). Examples of such reactions include the tricarboxylic acid cycle, synthesis of fatty acids and long-chain phospholipids, synthesis of alcohols and aldehydes, synthesis of intermediary metabolites, and reactions involved in the amino acid degradation
30 pathways. Some of these reactions require input of energy, usually in the form of conversion of ATP to either ADP or AMP and pyrophosphate.

In many cases, a carbon substrate is derived from a small molecule containing at least two carbon atoms. The carbon substrate is often covalently bound to a larger molecule which acts as a carbon substrate carrier molecule within the cell. In the biosynthetic mechanisms described above,

the carrier molecule is coenzyme A. Coenzyme A (CoA) is structurally related to derivatives of the nucleotide ADP and consists of 4'-phosphopantetheine linked via a phosphodiester bond to the alpha phosphate group of adenosine 3',5'-bisphosphate. The terminal thiol group of 4'-phosphopantetheine acts as the site for carbon substrate bond formation. The predominant carbon substrates which utilize

5 CoA as a carrier molecule during biosynthesis and intermediary metabolism in the cell are acetyl, succinyl, and propionyl moieties, collectively referred to as acyl groups. Other carbon substrates include enoyl lipid, which acts as a fatty acid oxidation intermediate, and carnitine, which acts as an acetyl-CoA flux regulator/ mitochondrial acyl group transfer protein. Acyl-CoA and acetyl-CoA are synthesized in the cell by acyl-CoA synthetase and acetyl-CoA synthetase, respectively.

10 Activation of fatty acids is mediated by at least three forms of acyl-CoA synthetase activity:

i) acetyl-CoA synthetase, which activates acetate and several other low molecular weight carboxylic acids and is found in muscle mitochondria and the cytosol of other tissues; ii) medium-chain acyl-CoA synthetase, which activates fatty acids containing between four and eleven carbon atoms (predominantly from dietary sources), and is present only in liver mitochondria; and iii) acyl CoA

15 synthetase, which is specific for long chain fatty acids with between six and twenty carbon atoms, and is found in microsomes and the mitochondria. Proteins associated with acyl-CoA synthetase activity have been identified from many sources including bacteria, yeast, plants, mouse, and man. The activity of acyl-CoA synthetase may be modulated by phosphorylation of the enzyme by cAMP-dependent protein kinase.

20 Ligases forming carbon-nitrogen bonds include amide synthases such as glutamine synthetase (glutamate-ammonia ligase) that catalyzes the amination of glutamic acid to glutamine by ammonia using the energy of ATP hydrolysis. Glutamine is the primary source for the amino group in various amide transfer reactions involved in de novo pyrimidine nucleotide synthesis and in purine and pyrimidine ribonucleotide interconversions. Overexpression of glutamine synthetase has been

25 observed in primary liver cancer (Christa, L. et al. (1994) Gastroent. 106:1312-1320).

Acid-amino-acid ligases (peptide synthases) are represented by the ubiquitin proteases which are associated with the ubiquitin conjugation system (UCS), a major pathway for the degradation of cellular proteins in eukaryotic cells and some bacteria. The UCS mediates the elimination of abnormal proteins and regulates the half-lives of important regulatory proteins that control cellular

30 processes such as gene transcription and cell cycle progression. In the UCS pathway, proteins targeted for degradation are conjugated to a ubiquitin (Ub), a small heat stable protein. Ub is first activated by a ubiquitin-activating enzyme (E1), and then transferred to one of several Ub-conjugating enzymes (E2). E2 then links the Ub molecule through its C-terminal glycine to an internal lysine (acceptor lysine) of a target protein. The ubiquitinated protein is then recognized and

degraded by proteasome, a large, multisubunit proteolytic enzyme complex, and ubiquitin is released for reutilization by ubiquitin protease. The UCS is implicated in the degradation of mitotic cyclic kinases, oncoproteins, tumor suppressor genes such as p53, viral proteins, cell surface receptors associated with signal transduction, transcriptional regulators, and mutated or damaged proteins
5 (Ciechanover, A. (1994) *Cell* 79:13-21). A murine proto-oncogene, Unp, encodes a nuclear ubiquitin protease whose overexpression leads to oncogenic transformation of NIH3T3 cells, and the human homolog of this gene is consistently elevated in small cell tumors and adenocarcinomas of the lung (Gray, D.A. (1995) *Oncogene* 10:2179-2183).

Cyclo-ligases and other carbon-nitrogen ligases comprise various enzymes and enzyme
10 complexes that participate in the de novo pathways to purine and pyrimidine biosynthesis. Because these pathways are critical to the synthesis of nucleotides for replication of both RNA and DNA, many of these enzymes have been the targets of clinical agents for the treatment of cell proliferative disorders such as cancer and infectious diseases.

Purine biosynthesis occurs de novo from the amino acids glycine and glutamine, and other
15 small molecules. Three of the key reactions in this process are catalyzed by a trifunctional enzyme composed of glycinamide-ribonucleotide synthetase (GARS), aminoimidazole ribonucleotide synthetase (AIRS), and glycinamide ribonucleotide transformylase (GART). Together these three enzymes combine ribosylamine phosphate with glycine to yield phosphoribosyl aminoimidazole, a precursor to both adenylate and guanylate nucleotides. This trifunctional protein has been implicated
20 in the pathology of Downs syndrome (Aimi, J. et al. (1990) *Nucleic Acid Res.* 18:6665-6672). Adenylosuccinate synthetase catalyzes a later step in purine biosynthesis that converts inosinic acid to adenylosuccinate, a key step on the path to ATP synthesis. This enzyme is also similar to another carbon-nitrogen ligase, argininosuccinate synthetase, that catalyzes a similar reaction in the urea cycle (Powell, S.M. et al. (1992) *FEBS Lett.* 303:4-10).

25 Like the de novo biosynthesis of purines, de novo synthesis of the pyrimidine nucleotides uridylate and cytidylate also arises from a common precursor, in this instance the nucleotide orotidylate derived from orotate and phosphoribosyl pyrophosphate (PPRP). Again a trifunctional enzyme comprising three carbon-nitrogen ligases plays a key role in the process. In this case the enzymes aspartate transcarbamylase (ATCase), carbamyl phosphate synthetase II, and dihydroorotase
30 (DHOase) are encoded by a single gene called CAD. Together these three enzymes combine the initial reactants in pyrimidine biosynthesis, glutamine, CO₂, and ATP to form dihydroorotate, the precursor to orotate and orotidylate (Iwahana, H. et al. (1996) *Biochem. Biophys. Res. Commun.* 219:249-255). Further steps then lead to the synthesis of uridine nucleotides from orotidylate. Cytidine nucleotides are derived from uridine-5'-triphosphate (UTP) by the amidation of UTP using

glutamine as the amino donor and the enzyme CTP synthetase. Regulatory mutations in the human CTP synthetase are believed to confer multi-drug resistance to agents widely used in cancer therapy (Yamauchi, M. et al. (1990) EMBO J. 9:2095-2099).

Ligases forming carbon-carbon bonds include the carboxylases acetyl-CoA carboxylase and pyruvate carboxylase. Acetyl-CoA carboxylase catalyzes the carboxylation of acetyl-CoA from CO₂ and H₂O using the energy of ATP hydrolysis. Acetyl-CoA carboxylase is the rate-limiting step in the biogenesis of long-chain fatty acids. Two isoforms of acetyl-CoA carboxylase, types I and types II, are expressed in human in a tissue-specific manner (Ha, J. et al. (1994) Eur. J. Biochem. 219:297-306). Pyruvate carboxylase is a nuclear-encoded mitochondrial enzyme that catalyzes the conversion of pyruvate to oxaloacetate, a key intermediate in the citric acid cycle.

Ligases forming phosphoric ester bonds include the DNA ligases involved in both DNA replication and repair. DNA ligases seal phosphodiester bonds between two adjacent nucleotides in a DNA chain using the energy from ATP hydrolysis to first activate the free 5'-phosphate of one nucleotide and then react it with the 3'-OH group of the adjacent nucleotide. This resealing reaction is used in both DNA replication to join small DNA fragments called Okazaki fragments that are transiently formed in the process of replicating new DNA, and in DNA repair. DNA repair is the process by which accidental base changes, such as those produced by oxidative damage, hydrolytic attack, or uncontrolled methylation of DNA, are corrected before replication or transcription of the DNA can occur. Bloom's syndrome is an inherited human disease in which individuals are partially deficient in DNA ligation and consequently have an increased incidence of cancer (Alberts, B. et al. (1994) The Molecular Biology of the Cell, Garland Publishing Inc., New York NY, p. 247).

Molecules Associated with Growth and Development

Human growth and development requires the spatial and temporal regulation of cell differentiation, cell proliferation, and apoptosis. These processes coordinately control reproduction, aging, embryogenesis, morphogenesis, organogenesis, and tissue repair and maintenance. At the cellular level, growth and development is governed by the cell's decision to enter into or exit from the cell division cycle and by the cell's commitment to a terminally differentiated state. These decisions are made by the cell in response to extracellular signals and other environmental cues it receives. The following discussion focuses on the molecular mechanisms of cell division, reproduction, cell differentiation and proliferation, apoptosis, and aging.

Cell Division

Cell division is the fundamental process by which all living things grow and reproduce. In unicellular organisms such as yeast and bacteria, each cell division doubles the number of organisms,

while in multicellular species many rounds of cell division are required to replace cells lost by wear or by programmed cell death, and for cell differentiation to produce a new tissue or organ. Details of the cell division cycle may vary, but the basic process consists of three principle events. The first event, interphase, involves preparations for cell division, replication of the DNA, and production of essential proteins. In the second event, mitosis, the nuclear material is divided and separates to opposite sides of the cell. The final event, cytokinesis, is division and fission of the cell cytoplasm. The sequence and timing of cell cycle transitions is under the control of the cell cycle regulation system which controls the process by positive or negative regulatory circuits at various check points.

Regulated progression of the cell cycle depends on the integration of growth control pathways with the basic cell cycle machinery. Cell cycle regulators have been identified by selecting for human and yeast cDNAs that block or activate cell cycle arrest signals in the yeast mating pheromone pathway when they are overexpressed. Known regulators include human CPR (cell cycle progression restoration) genes, such as CPR8 and CPR2, and yeast CDC (cell division control) genes, including CDC91, that block the arrest signals. The CPR genes express a variety of proteins including cyclins, tumor suppressor binding proteins, chaperones, transcription factors, translation factors, and RNA-binding proteins (Edwards, M.C. et al.(1997) Genetics 147:1063-1076).

Several cell cycle transitions, including the entry and exit of a cell from mitosis, are dependent upon the activation and inhibition of cyclin-dependent kinases (Cdks). The Cdks are composed of a kinase subunit, Cdk, and an activating subunit, cyclin, in a complex that is subject to many levels of regulation. There appears to be a single Cdk in Saccharomyces cerevisiae and Saccharomyces pombe whereas mammals have a variety of specialized Cdks. Cyclins act by binding to and activating cyclin-dependent protein kinases which then phosphorylate and activate selected proteins involved in the mitotic process. The Cdk-cyclin complex is both positively and negatively regulated by phosphorylation, and by targeted degradation involving molecules such as CDC4 and CDC53. In addition, Cdks are further regulated by binding to inhibitors and other proteins such as Suc1 that modify their specificity or accessibility to regulators (Patra, D. and W.G. Dunphy (1996) Genes Dev. 10:1503-1515; and Mathias, N. et al. (1996) Mol. Cell Biol. 16:6634-6643).

Reproduction

The male and female reproductive systems are complex and involve many aspects of growth and development. The anatomy and physiology of the male and female reproductive systems are reviewed in (Guyton, A.C. (1991) Textbook of Medical Physiology, W.B. Saunders Co., Philadelphia PA, pp. 899-928).

The male reproductive system includes the process of spermatogenesis, in which the sperm are formed, and male reproductive functions are regulated by various hormones and their effects on

accessory sexual organs, cellular metabolism, growth, and other bodily functions.

Spermatogenesis begins at puberty as a result of stimulation by gonadotropic hormones released from the anterior pituitary. Immature sperm (spermatogonia) undergo several mitotic cell divisions before undergoing meiosis and full maturation. The testes secrete several male sex hormones, the most abundant being testosterone, that is essential for growth and division of the immature sperm, and for the masculine characteristics of the male body. Three other male sex hormones, gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) control sexual function.

The uterus, ovaries, fallopian tubes, vagina, and breasts comprise the female reproductive system. The ovaries and uterus are the source of ova and the location of fetal development, respectively. The fallopian tubes and vagina are accessory organs attached to the top and bottom of the uterus, respectively. Both the uterus and ovaries have additional roles in the development and loss of reproductive capability during a female's lifetime. The primary role of the breasts is lactation. Multiple endocrine signals from the ovaries, uterus, pituitary, hypothalamus, adrenal glands, and other tissues coordinate reproduction and lactation. These signals vary during the monthly menstruation cycle and during the female's lifetime. Similarly, the sensitivity of reproductive organs to these endocrine signals varies during the female's lifetime.

A combination of positive and negative feedback to the ovaries, pituitary and hypothalamus glands controls physiologic changes during the monthly ovulation and endometrial cycles. The anterior pituitary secretes two major gonadotropin hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), regulated by negative feedback of steroids, most notably by ovarian estradiol. If fertilization does not occur, estrogen and progesterone levels decrease. This sudden reduction of the ovarian hormones leads to menstruation, the desquamation of the endometrium.

Hormones further govern all the steps of pregnancy, parturition, lactation, and menopause. During pregnancy large quantities of human chorionic gonadotropin (hCG), estrogens, progesterone, and human chorionic somatomammotropin (hCS) are formed by the placenta. hCG, a glycoprotein similar to luteinizing hormone, stimulates the corpus luteum to continue producing more progesterone and estrogens, rather than to involute as occurs if the ovum is not fertilized. hCS is similar to growth hormone and is crucial for fetal nutrition.

The female breast also matures during pregnancy. Large amounts of estrogen secreted by the placenta trigger growth and branching of the breast milk ductal system while lactation is initiated by the secretion of prolactin by the pituitary gland.

Parturition involves several hormonal changes that increase uterine contractility toward the end of pregnancy, as follows. The levels of estrogens increase more than those of progesterone. Oxytocin

is secreted by the neurohypophysis. Concomitantly, uterine sensitivity to oxytocin increases. The fetus itself secretes oxytocin, cortisol (from adrenal glands), and prostaglandins.

Menopause occurs when most of the ovarian follicles have degenerated. The ovary then produces less estradiol, reducing the negative feedback on the pituitary and hypothalamus glands.

- 5 Mean levels of circulating FSH and LH increase, even as ovulatory cycles continue. Therefore, the ovary is less responsive to gonadotropins, and there is an increase in the time between menstrual cycles. Consequently, menstrual bleeding ceases and reproductive capability ends.

Cell Differentiation and Proliferation

- 10 Tissue growth involves complex and ordered patterns of cell proliferation, cell differentiation, and apoptosis. Cell proliferation must be regulated to maintain both the number of cells and their spatial organization. This regulation depends upon the appropriate expression of proteins which control cell cycle progression in response to extracellular signals, such as growth factors and other mitogens, and intracellular cues, such as DNA damage or nutrient starvation. Molecules which directly or indirectly modulate cell cycle progression fall into several categories, including growth factors and their
- 15 receptors, second messenger and signal transduction proteins, oncogene products, tumor-suppressor proteins, and mitosis-promoting factors.

- Growth factors were originally described as serum factors required to promote cell proliferation. Most growth factors are large, secreted polypeptides that act on cells in their local environment. Growth factors bind to and activate specific cell surface receptors and initiate
- 20 intracellular signal transduction cascades. Many growth factor receptors are classified as receptor tyrosine kinases which undergo autophosphorylation upon ligand binding. Autophosphorylation enables the receptor to interact with signal transduction proteins characterized by the presence of SH2 or SH3 domains (Src homology regions 2 or 3). These proteins then modulate the activity state of small G-proteins, such as Ras, Rab, and Rho, along with GTPase activating proteins (GAPs), guanine
- 25 nucleotide releasing proteins (GNRPs), and other guanine nucleotide exchange factors. Small G proteins act as molecular switches that activate other downstream events, such as mitogen-activated protein kinase (MAP kinase) cascades. MAP kinases ultimately activate transcription of mitosis-promoting genes.

- In addition to growth factors, small signaling peptides and hormones also influence cell
- 30 proliferation. These molecules bind primarily to another class of receptor, the trimeric G-protein coupled receptor (GPCR), found predominantly on the surface of immune, neuronal and neuroendocrine cells. Upon ligand binding, the GPCR activates a trimeric G protein which in turn triggers increased levels of intracellular second messengers such as phospholipase C, Ca²⁺, and cyclic AMP. Most GPCR-mediated signaling pathways indirectly promote cell proliferation by causing the secretion or

breakdown of other signaling molecules that have direct mitogenic effects. These signaling cascades often involve activation of kinases and phosphatases. Some growth factors, such as some members of the transforming growth factor beta (TGF- β) family, act on some cells to stimulate cell proliferation and on other cells to inhibit it. Growth factors may also stimulate a cell at one concentration and inhibit
5 the same cell at another concentration. Most growth factors also have a multitude of other actions besides the regulation of cell growth and division: they can control the proliferation, survival, differentiation, migration, or function of cells depending on the circumstance. For example, the tumor necrosis factor/nerve growth factor (TNF/NGF) family can activate or inhibit cell death, as well as regulate proliferation and differentiation. The cell response depends on the type of cell, its stage of
10 differentiation and transformation status, which surface receptors are stimulated, and the types of stimuli acting on the cell (Smith, A. et al. (1994) *Cell* 76:959-962; and Nocentini, G. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94:6216-6221).

Neighboring cells in a tissue compete for growth factors, and when provided with "unlimited" quantities in a perfused system will grow to even higher cell densities before reaching density-dependent
15 inhibition of cell division. Cells often demonstrate an anchorage dependence of cell division as well. This anchorage dependence may be associated with the formation of focal contacts linking the cytoskeleton with the extracellular matrix (ECM). The expression of ECM components can be stimulated by growth factors. For example, TGF- β stimulates fibroblasts to produce a variety of ECM proteins, including fibronectin, collagen, and tenascin (Pearson, C.A. et al. (1988) *EMBO J.* 7:2677-
20 2981). In fact, for some cell types specific ECM molecules, such as laminin or fibronectin, may act as growth factors. Tenascin-C and -R, expressed in developing and lesioned neural tissue, provide stimulatory/anti-adhesive or inhibitory properties, respectively, for axonal growth (Faissner, A. (1997) *Cell Tissue Res.* 290:331-341).

Cancers are associated with the activation of oncogenes which are derived from normal cellular
25 genes. These oncogenes encode oncoproteins which convert normal cells into malignant cells. Some oncoproteins are mutant isoforms of the normal protein, and other oncoproteins are abnormally expressed with respect to location or amount of expression. The latter category of oncoprotein causes cancer by altering transcriptional control of cell proliferation. Five classes of oncoproteins are known to affect cell cycle controls. These classes include growth factors, growth factor receptors, intracellular
30 signal transducers, nuclear transcription factors, and cell-cycle control proteins. Viral oncogenes are integrated into the human genome after infection of human cells by certain viruses. Examples of viral oncogenes include v-src, v-abl, and v-fps.

Many oncogenes have been identified and characterized. These include sis, erbA, erbB, her-2, mutated G_s, src, abl, ras, crk, jun, fos, myc, and mutated tumor-suppressor genes such as RB, p53,

mdm2, Cip1, p16, and cyclin D. Transformation of normal genes to oncogenes may also occur by chromosomal translocation. The Philadelphia chromosome, characteristic of chronic myeloid leukemia and a subset of acute lymphoblastic leukemias, results from a reciprocal translocation between chromosomes 9 and 22 that moves a truncated portion of the proto-oncogene c-abl to the breakpoint cluster region (bcr) on chromosome 22.

Tumor-suppressor genes are involved in regulating cell proliferation. Mutations which cause reduced or loss of function in tumor-suppressor genes result in uncontrolled cell proliferation. For example, the retinoblastoma gene product (RB), in a non-phosphorylated state, binds several early-response genes and suppresses their transcription, thus blocking cell division. Phosphorylation of RB causes it to dissociate from the genes, releasing the suppression, and allowing cell division to proceed.

Apoptosis

Apoptosis is the genetically controlled process by which unneeded or defective cells undergo programmed cell death. Selective elimination of cells is as important for morphogenesis and tissue remodeling as is cell proliferation and differentiation. Lack of apoptosis may result in hyperplasia and other disorders associated with increased cell proliferation. Apoptosis is also a critical component of the immune response. Immune cells such as cytotoxic T-cells and natural killer cells prevent the spread of disease by inducing apoptosis in tumor cells and virus-infected cells. In addition, immune cells that fail to distinguish self molecules from foreign molecules must be eliminated by apoptosis to avoid an autoimmune response.

Apoptotic cells undergo distinct morphological changes. Hallmarks of apoptosis include cell shrinkage, nuclear and cytoplasmic condensation, and alterations in plasma membrane topology. Biochemically, apoptotic cells are characterized by increased intracellular calcium concentration, fragmentation of chromosomal DNA, and expression of novel cell surface components.

The molecular mechanisms of apoptosis are highly conserved, and many of the key protein regulators and effectors of apoptosis have been identified. Apoptosis generally proceeds in response to a signal which is transduced intracellularly and results in altered patterns of gene expression and protein activity. Signaling molecules such as hormones and cytokines are known both to stimulate and to inhibit apoptosis through interactions with cell surface receptors. Transcription factors also play an important role in the onset of apoptosis. A number of downstream effector molecules, particularly proteases such as the cysteine proteases called caspases, have been implicated in the degradation of cellular components and the proteolytic activation of other apoptotic effectors.

Aging and Senescence

Studies of the aging process or senescence have shown a number of characteristic cellular and molecular changes (Fauci et al. (1998) Harrison's Principles of Internal Medicine, McGraw-Hill, New

York NY, p.37). These characteristics include increases in chromosome structural abnormalities, DNA cross-linking, incidence of single-stranded breaks in DNA, losses in DNA methylation, and degradation of telomere regions. In addition to these DNA changes, post-translational alterations of proteins increase including, deamidation, oxidation, cross-linking, and nonenzymatic glycation. Still further
5 molecular changes occur in the mitochondria of aging cells through deterioration of structure. These changes eventually contribute to decreased function in every organ of the body.

Biochemical Pathway Molecules

Biochemical pathways are responsible for regulating metabolism, growth and development,
10 protein secretion and trafficking, environmental responses, and ecological interactions including immune response and response to parasites.

DNA replication

Deoxyribonucleic acid (DNA), the genetic material, is found in both the nucleus and mitochondria of human cells. The bulk of human DNA is nuclear, in the form of linear chromosomes,
15 while mitochondrial DNA is circular. DNA replication begins at specific sites called origins of replication. Bidirectional synthesis occurs from the origin via two growing forks that move in opposite directions. Replication is semi-conservative, with each daughter duplex containing one old strand and its newly synthesized complementary partner. Proteins involved in DNA replication include DNA polymerases, DNA primase, telomerase, DNA helicase, topoisomerases, DNA ligases, replication
20 factors, and DNA-binding proteins.

DNA Recombination and Repair

Cells are constantly faced with replication errors and environmental assault (such as ultraviolet irradiation) that can produce DNA damage. Damage to DNA consists of any change that modifies the structure of the molecule. Changes to DNA can be divided into two general classes, single base
25 changes and structural distortions. Any damage to DNA can produce a mutation, and the mutation may produce a disorder, such as cancer.

Changes in DNA are recognized by repair systems within the cell. These repair systems act to correct the damage and thus prevent any deleterious affects of a mutational event. Repair systems can be divided into three general types, direct repair, excision repair, and retrieval systems. Proteins
30 involved in DNA repair include DNA polymerase, excision repair proteins, excision and cross link repair proteins, recombination and repair proteins, RAD51 proteins, and BLN and WRN proteins that are homologs of RecQ helicase. When the repair systems are eliminated, cells become exceedingly sensitive to environmental mutagens, such as ultraviolet irradiation. Patients with disorders associated with a loss in DNA repair systems often exhibit a high sensitivity to environmental mutagens.

Examples of such disorders include xeroderma pigmentosum (XP), Bloom's syndrome (BS), and Werner's syndrome (WS) (Yamagata, K. et al. (1998) Proc. Natl. Acad. Sci. USA 95:8733-8738), ataxia telangiectasia, Cockayne's syndrome, and Fanconi's anemia.

Recombination is the process whereby new DNA sequences are generated by the movements of large pieces of DNA. In homologous recombination, which occurs during meiosis and DNA repair, parent DNA duplexes align at regions of sequence similarity, and new DNA molecules form by the breakage and joining of homologous segments. Proteins involved include RAD51 recombinase. In site-specific recombination, two specific but not necessarily homologous DNA sequences are exchanged. In the immune system this process generates a diverse collection of antibody and T cell receptor genes. Proteins involved in site-specific recombination in the immune system include recombination activating genes 1 and 2 (RAG1 and RAG2). A defect in immune system site-specific recombination causes severe combined immunodeficiency disease in mice.

RNA Metabolism

Ribonucleic acid (RNA) is a linear single-stranded polymer of four nucleotides, ATP, CTP, UTP, and GTP. In most organisms, RNA is transcribed as a copy of DNA, the genetic material of the organism. In retroviruses RNA rather than DNA serves as the genetic material. RNA copies of the genetic material encode proteins or serve various structural, catalytic, or regulatory roles in organisms. RNA is classified according to its cellular localization and function. Messenger RNAs (mRNAs) encode polypeptides. Ribosomal RNAs (rRNAs) are assembled, along with ribosomal proteins, into ribosomes, which are cytoplasmic particles that translate mRNA into polypeptides. Transfer RNAs (tRNAs) are cytosolic adaptor molecules that function in mRNA translation by recognizing both an mRNA codon and the amino acid that matches that codon. Heterogeneous nuclear RNAs (hnRNAs) include mRNA precursors and other nuclear RNAs of various sizes. Small nuclear RNAs (snRNAs) are a part of the nuclear spliceosome complex that removes intervening, non-coding sequences (introns) and rejoins exons in pre-mRNAs.

RNA Transcription

The transcription process synthesizes an RNA copy of DNA. Proteins involved include multi-subunit RNA polymerases, transcription factors IIA, IIB, IID, IIE, IIF, IIH, and IIJ. Many transcription factors incorporate DNA-binding structural motifs which comprise either α -helices or β -sheets that bind to the major groove of DNA. Four well-characterized structural motifs are helix-turn-helix, zinc finger, leucine zipper, and helix-loop-helix.

RNA Processing

Various proteins are necessary for processing of transcribed RNAs in the nucleus. Pre-mRNA processing steps include capping at the 5' end with methylguanosine, polyadenylating the 3' end, and

splicing to remove introns. The spliceosomal complex is comprised of five small nuclear ribonucleoprotein particles (snRNPs) designated U1, U2, U4, U5, and U6. Each snRNP contains a single species of snRNA and about ten proteins. The RNA components of some snRNPs recognize and base-pair with intron consensus sequences. The protein components mediate spliceosome assembly and the splicing reaction. Autoantibodies to snRNP proteins are found in the blood of patients with systemic lupus erythematosus (Stryer, L. (1995) Biochemistry W.H. Freeman and Company, New York NY, p. 863).

Heterogeneous nuclear ribonucleoproteins (hnRNPs) have been identified that have roles in splicing, exporting of the mature RNAs to the cytoplasm, and mRNA translation (Biamonti, G. et al. (1998) Clin. Exp. Rheumatol. 16:317-326). Some examples of hnRNPs include the yeast proteins Hrp1p, involved in cleavage and polyadenylation at the 3' end of the RNA; Cbp80p, involved in capping the 5' end of the RNA; and Npl3p, a homolog of mammalian hnRNP A1, involved in export of mRNA from the nucleus (Shen, E.C. et al. (1998) Genes Dev. 12:679-691). HnRNPs have been shown to be important targets of the autoimmune response in rheumatic diseases (Biamonti, supra).

Many snRNP proteins, hnRNP proteins, and alternative splicing factors are characterized by an RNA recognition motif (RRM). (Reviewed in Birney, E. et al. (1993) Nucleic Acids Res. 21:5803-5816.) The RRM is about 80 amino acids in length and forms four β -strands and two α -helices arranged in an α/β sandwich. The RRM contains a core RNP-1 octapeptide motif along with surrounding conserved sequences.

RNA Stability and Degradation

RNA helicases alter and regulate RNA conformation and secondary structure by using energy derived from ATP hydrolysis to destabilize and unwind RNA duplexes. The most well-characterized and ubiquitous family of RNA helicases is the DEAD-box family, so named for the conserved B-type ATP-binding motif which is diagnostic of proteins in this family. Over 40 DEAD-box helicases have been identified in organisms as diverse as bacteria, insects, yeast, amphibians, mammals, and plants. DEAD-box helicases function in diverse processes such as translation initiation, splicing, ribosome assembly, and RNA editing, transport, and stability. Some DEAD-box helicases play tissue- and stage-specific roles in spermatogenesis and embryogenesis. (Reviewed in Linder, P. et al. (1989) Nature 337:121-122.)

Overexpression of the DEAD-box 1 protein (DDX1) may play a role in the progression of neuroblastoma (Nb) and retinoblastoma (Rb) tumors. Other DEAD-box helicases have been implicated either directly or indirectly in ultraviolet light-induced tumors, B cell lymphoma, and myeloid malignancies. (Reviewed in Godbout, R. et al. (1998) J. Biol. Chem. 273:21161-21168.)

Ribonucleases (RNases) catalyze the hydrolysis of phosphodiester bonds in RNA chains, thus

cleaving the RNA. For example, RNase P is a ribonucleoprotein enzyme which cleaves the 5' end of pre-tRNAs as part of their maturation process. RNase H digests the RNA strand of an RNA/DNA hybrid. Such hybrids occur in cells invaded by retroviruses, and RNase H is an important enzyme in the retroviral replication cycle. RNase H domains are often found as a domain associated with reverse transcriptases. RNase activity in serum and cell extracts is elevated in a variety of cancers and infectious diseases (Schein, C.H. (1997) Nat. Biotechnol. 15:529-536). Regulation of RNase activity is being investigated as a means to control tumor angiogenesis, allergic reactions, viral infection and replication, and fungal infections.

Protein Translation

The eukaryotic ribosome is composed of a 60S (large) subunit and a 40S (small) subunit, which together form the 80S ribosome. In addition to the 18S, 28S, 5S, and 5.8S rRNAs, the ribosome also contains more than fifty proteins. The ribosomal proteins have a prefix which denotes the subunit to which they belong, either L (large) or S (small). Three important sites are identified on the ribosome. The aminoacyl-tRNA site (A site) is where charged tRNAs (with the exception of the initiator-tRNA) bind on arrival at the ribosome. The peptidyl-tRNA site (P site) is where new peptide bonds are formed, as well as where the initiator tRNA binds. The exit site (E site) is where deacylated tRNAs bind prior to their release from the ribosome. (Translation is reviewed in Stryer, L. (1995)

Biochemistry, W.H. Freeman and Company, New York NY, pp. 875-908; and Lodish, H. et al. (1995) Molecular Cell Biology, Scientific American Books, New York NY, pp. 119-138.)

tRNA Charging

Protein biosynthesis depends on each amino acid forming a linkage with the appropriate tRNA. The aminoacyl-tRNA synthetases are responsible for the activation and correct attachment of an amino acid with its cognate tRNA. The 20 aminoacyl-tRNA synthetase enzymes can be divided into two structural classes, Class I and Class II. Autoantibodies against aminoacyl-tRNAs are generated by patients with dermatomyositis and polymyositis, and correlate strongly with complicating interstitial lung disease (ILD). These antibodies appear to be generated in response to viral infection, and coxsackie virus has been used to induce experimental viral myositis in animals.

Translation Initiation

Initiation of translation can be divided into three stages. The first stage brings an initiator transfer RNA (Met-tRNA_i) together with the 40S ribosomal subunit to form the 43S preinitiation complex. The second stage binds the 43S preinitiation complex to the mRNA, followed by migration of the complex to the correct AUG initiation codon. The third stage brings the 60S ribosomal subunit to the 40S subunit to generate an 80S ribosome at the initiation codon. Regulation of translation primarily involves the first and second stage in the initiation process (Pain, V.M. (1996) Eur. J. Biochem.

236:747-771).

Several initiation factors, many of which contain multiple subunits, are involved in bringing an initiator tRNA and 40S ribosomal subunit together. eIF2, a guanine nucleotide binding protein, recruits the initiator tRNA to the 40S ribosomal subunit. Only when eIF2 is bound to GTP does it associate
 5 with the initiator tRNA. eIF2B, a guanine nucleotide exchange protein, is responsible for converting eIF2 from the GDP-bound inactive form to the GTP-bound active form. Two other factors, eIF1A and eIF3 bind and stabilize the 40S subunit by interacting with 18S ribosomal RNA and specific ribosomal structural proteins. eIF3 is also involved in association of the 40S ribosomal subunit with mRNA. The Met-tRNA_i, eIF1A, eIF3, and 40S ribosomal subunit together make up the 43S preinitiation complex
 10 (Pain, *supra*).

Additional factors are required for binding of the 43S preinitiation complex to an mRNA molecule, and the process is regulated at several levels. eIF4F is a complex consisting of three proteins: eIF4E, eIF4A, and eIF4G. eIF4E recognizes and binds to the mRNA 5'-terminal m⁷GTP cap, eIF4A is a bidirectional RNA-dependent helicase, and eIF4G is a scaffolding polypeptide. eIF4G has three
 15 binding domains. The N-terminal third of eIF4G interacts with eIF4E, the central third interacts with eIF4A, and the C-terminal third interacts with eIF3 bound to the 43S preinitiation complex. Thus, eIF4G acts as a bridge between the 40S ribosomal subunit and the mRNA (Hentze, M.W. (1997) Science 275:500-501).

The ability of eIF4F to initiate binding of the 43S preinitiation complex is regulated by
 20 structural features of the mRNA. The mRNA molecule has an untranslated region (UTR) between the 5' cap and the AUG start codon. In some mRNAs this region forms secondary structures that impede binding of the 43S preinitiation complex. The helicase activity of eIF4A is thought to function in removing this secondary structure to facilitate binding of the 43S preinitiation complex (Pain, *supra*).

Translation Elongation

25 Elongation is the process whereby additional amino acids are joined to the initiator methionine to form the complete polypeptide chain. The elongation factors EF1 α , EF1 β γ , and EF2 are involved in elongating the polypeptide chain following initiation. EF1 α is a GTP-binding protein. In EF1 α 's GTP-bound form, it brings an aminoacyl-tRNA to the ribosome's A site. The amino acid attached to the newly arrived aminoacyl-tRNA forms a peptide bond with the initiator methionine. The GTP on
 30 EF1 α is hydrolyzed to GDP, and EF1 α -GDP dissociates from the ribosome. EF1 β γ binds EF1 α -GDP and induces the dissociation of GDP from EF1 α , allowing EF1 α to bind GTP and a new cycle to begin.

As subsequent aminoacyl-tRNAs are brought to the ribosome, EF-G, another GTP-binding protein, catalyzes the translocation of tRNAs from the A site to the P site and finally to the E site of the ribosome. This allows the processivity of translation.

Translation Termination

The release factor eRF carries out termination of translation. eRF recognizes stop codons in the mRNA, leading to the release of the polypeptide chain from the ribosome.

Post-Translational Pathways

5 Proteins may be modified after translation by the addition of phosphate, sugar, prenyl, fatty acid, and other chemical groups. These modifications are often required for proper protein activity. Enzymes involved in post-translational modification include kinases, phosphatases, glycosyltransferases, and prenyltransferases. The conformation of proteins may also be modified after translation by the introduction and rearrangement of disulfide bonds (rearrangement catalyzed by
10 protein disulfide isomerase), the isomerization of proline sidechains by prolyl isomerase, and by interactions with molecular chaperone proteins.

Proteins may also be cleaved by proteases. Such cleavage may result in activation, inactivation, or complete degradation of the protein. Proteases include serine proteases, cysteine proteases, aspartic proteases, and metalloproteases. Signal peptidase in the endoplasmic reticulum
15 (ER) lumen cleaves the signal peptide from membrane or secretory proteins that are imported into the ER. Ubiquitin proteases are associated with the ubiquitin conjugation system (UCS), a major pathway for the degradation of cellular proteins in eukaryotic cells and some bacteria. The UCS mediates the elimination of abnormal proteins and regulates the half-lives of important regulatory proteins that control cellular processes such as gene transcription and cell cycle progression. In the
20 UCS pathway, proteins targeted for degradation are conjugated to a ubiquitin, a small heat stable protein. Proteins involved in the UCS include ubiquitin-activating enzyme, ubiquitin-conjugating enzymes, ubiquitin-ligases, and ubiquitin C-terminal hydrolases. The ubiquitinated protein is then recognized and degraded by the proteasome, a large, multisubunit proteolytic enzyme complex, and ubiquitin is released for reutilization by ubiquitin protease.

Lipid Metabolism

25 Lipids are water-insoluble, oily or greasy substances that are soluble in nonpolar solvents such as chloroform or ether. Neutral fats (triacylglycerols) serve as major fuels and energy stores. Polar lipids, such as phospholipids, sphingolipids, glycolipids, and cholesterol, are key structural components of cell membranes.

30 Lipid metabolism is involved in human diseases and disorders. In the arterial disease atherosclerosis, fatty lesions form on the inside of the arterial wall. These lesions promote the loss of arterial flexibility and the formation of blood clots (Guyton, A.C. Textbook of Medical Physiology (1991) W.B. Saunders Company, Philadelphia PA, pp.760-763). In Tay-Sachs disease, the GM₂ ganglioside (a sphingolipid) accumulates in lysosomes of the central nervous system due to a lack of the

enzyme N-acetylhexosaminidase. Patients suffer nervous system degeneration leading to early death (Fauci, A.S. et al. (1998) Harrison's Principles of Internal Medicine McGraw-Hill, New York NY, p. 2171). The Niemann-Pick diseases are caused by defects in lipid metabolism. Niemann-Pick diseases types A and B are caused by accumulation of sphingomyelin (a sphingolipid) and other lipids in the

5 central nervous system due to a defect in the enzyme sphingomyelinase, leading to neurodegeneration and lung disease. Niemann-Pick disease type C results from a defect in cholesterol transport, leading to the accumulation of sphingomyelin and cholesterol in lysosomes and a secondary reduction in sphingomyelinase activity. Neurological symptoms such as grand mal seizures, ataxia, and loss of

10 previously learned speech, manifest 1-2 years after birth. A mutation in the NPC protein, which contains a putative cholesterol-sensing domain, was found in a mouse model of Niemann-Pick disease type C (Fauci, supra, p. 2175; Loftus, S.K. et al. (1997) *Science* 277:232-235). (Lipid metabolism is reviewed in Stryer, L. (1995) Biochemistry, W.H. Freeman and Company, New York NY; Lehninger, A. (1982) Principles of Biochemistry Worth Publishers, Inc., New York NY; and ExPASy "Biochemical Pathways" index of Boehringer Mannheim World Wide Web site.)

15 Fatty Acid Synthesis

Fatty acids are long-chain organic acids with a single carboxyl group and a long non-polar hydrocarbon tail. Long-chain fatty acids are essential components of glycolipids, phospholipids, and cholesterol, which are building blocks for biological membranes, and of triglycerides, which are biological fuel molecules. Long-chain fatty acids are also substrates for eicosanoid production, and are

20 important in the functional modification of certain complex carbohydrates and proteins. 16-carbon and 18-carbon fatty acids are the most common.

Fatty acid synthesis occurs in the cytoplasm. In the first step, acetyl-Coenzyme A (CoA) carboxylase (ACC) synthesizes malonyl-CoA from acetyl-CoA and bicarbonate. The enzymes which catalyze the remaining reactions are covalently linked into a single polypeptide chain, referred to as the

25 multifunctional enzyme fatty acid synthase (FAS). FAS catalyzes the synthesis of palmitate from acetyl-CoA and malonyl-CoA. FAS contains acetyl transferase, malonyl transferase, β -ketoacetyl synthase, acyl carrier protein, β -ketoacyl reductase, dehydratase, enoyl reductase, and thioesterase activities. The final product of the FAS reaction is the 16-carbon fatty acid palmitate. Further elongation, as well as unsaturation, of palmitate by accessory enzymes of the ER produces the variety

30 of long chain fatty acids required by the individual cell. These enzymes include a NADH-cytochrome b_5 reductase, cytochrome b_5 , and a desaturase.

Phospholipid and Triacylglycerol Synthesis

Triacylglycerols, also known as triglycerides and neutral fats, are major energy stores in animals. Triacylglycerols are esters of glycerol with three fatty acid chains. Glycerol-3-phosphate is

produced from dihydroxyacetone phosphate by the enzyme glycerol phosphate dehydrogenase or from glycerol by glycerol kinase. Fatty acid-CoA's are produced from fatty acids by fatty acyl-CoA synthetases. Glycerol-3-phosphate is acylated with two fatty acyl-CoA's by the enzyme glycerol phosphate acyltransferase to give phosphatidate. Phosphatidate phosphatase converts phosphatidate to diacylglycerol, which is subsequently acylated to a triacylglycerol by the enzyme diglyceride acyltransferase. Phosphatidate phosphatase and diglyceride acyltransferase form a triacylglycerol synthetase complex bound to the ER membrane.

A major class of phospholipids are the phosphoglycerides, which are composed of a glycerol backbone, two fatty acid chains, and a phosphorylated alcohol. Phosphoglycerides are components of cell membranes. Principal phosphoglycerides are phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, and diphosphatidyl glycerol. Many enzymes involved in phosphoglyceride synthesis are associated with membranes (Meyers, R.A. (1995) Molecular Biology and Biotechnology, VCH Publishers Inc., New York NY, pp. 494-501). Phosphatidate is converted to CDP-diacylglycerol by the enzyme phosphatidate cytidylyltransferase (ExPASy ENZYME EC 2.7.7.41). Transfer of the diacylglycerol group from CDP-diacylglycerol to serine to yield phosphatidyl serine, or to inositol to yield phosphatidyl inositol, is catalyzed by the enzymes CDP-diacylglycerol-serine O-phosphatidyltransferase and CDP-diacylglycerol-inositol 3-phosphatidyltransferase, respectively (ExPASy ENZYME EC 2.7.8.8; ExPASy ENZYME EC 2.7.8.11). The enzyme phosphatidyl serine decarboxylase catalyzes the conversion of phosphatidyl serine to phosphatidyl ethanolamine, using a pyruvate cofactor (Voelker, D.R. (1997) *Biochim. Biophys. Acta* 1348:236-244). Phosphatidyl choline is formed using diet-derived choline by the reaction of CDP-choline with 1,2-diacylglycerol, catalyzed by diacylglycerol cholinephosphotransferase (ExPASy ENZYME 2.7.8.2).

Sterol, Steroid, and Isoprenoid Metabolism

Cholesterol, composed of four fused hydrocarbon rings with an alcohol at one end, moderates the fluidity of membranes in which it is incorporated. In addition, cholesterol is used in the synthesis of steroid hormones such as cortisol, progesterone, estrogen, and testosterone. Bile salts derived from cholesterol facilitate the digestion of lipids. Cholesterol in the skin forms a barrier that prevents excess water evaporation from the body. Farnesyl and geranylgeranyl groups, which are derived from cholesterol biosynthesis intermediates, are post-translationally added to signal transduction proteins such as ras and protein-targeting proteins such as rab. These modifications are important for the activities of these proteins (Guyton, supra; Stryer, supra, pp. 279-280, 691-702, 934).

Mammals obtain cholesterol derived from both de novo biosynthesis and the diet. The liver is the major site of cholesterol biosynthesis in mammals. Two acetyl-CoA molecules initially condense to form acetoacetyl-CoA, catalyzed by a thiolase. Acetoacetyl-CoA condenses with a third acetyl-CoA to

form hydroxymethylglutaryl-CoA (HMG-CoA), catalyzed by HMG-CoA synthase. Conversion of HMG-CoA to cholesterol is accomplished via a series of enzymatic steps known as the mevalonate pathway. The rate-limiting step is the conversion of HMG-CoA to mevalonate by HMG-CoA reductase. The drug lovastatin, a potent inhibitor of HMG-CoA reductase, is given to patients to
5 reduce their serum cholesterol levels. Other mevalonate pathway enzymes include mevalonate kinase, phosphomevalonate kinase, diphosphomevalonate decarboxylase, isopentenyl diphosphate isomerase, dimethylallyl transferase, geranyl transferase, farnesyl-diphosphate farnesyltransferase, squalene monooxygenase, lanosterol synthase, lanosterol oxidase, and 7-dehydrocholesterol reductase.

Cholesterol is used in the synthesis of steroid hormones such as cortisol, progesterone,
10 aldosterone, estrogen, and testosterone. First, cholesterol is converted to pregnenolone by cholesterol monooxygenases. The other steroid hormones are synthesized from pregnenolone by a series of enzyme-catalyzed reactions including oxidations, isomerizations, hydroxylations, reductions, and demethylations. Examples of these enzymes include steroid Δ -isomerase, 3β -hydroxy- Δ^5 -steroid dehydrogenase, steroid 21-monooxygenase, steroid 19-hydroxylase, and 3β -hydroxysteroid
15 dehydrogenase. Cholesterol is also the precursor to vitamin D.

Numerous compounds contain 5-carbon isoprene units derived from the mevalonate pathway intermediate isopentenyl pyrophosphate. Isoprenoid groups are found in vitamin K, ubiquinone, retinal, dolichol phosphate (a carrier of oligosaccharides needed for N-linked glycosylation), and farnesyl and geranylgeranyl groups that modify proteins. Enzymes involved include farnesyl transferase, polyprenyl
20 transferases, dolichyl phosphatase, and dolichyl kinase.

Sphingolipid Metabolism

Sphingolipids are an important class of membrane lipids that contain sphingosine, a long chain amino alcohol. They are composed of one long-chain fatty acid, one polar head alcohol, and sphingosine or sphingosine derivative. The three classes of sphingolipids are sphingomyelins,
25 cerebroside, and gangliosides. Sphingomyelins, which contain phosphocholine or phosphoethanolamine as their head group, are abundant in the myelin sheath surrounding nerve cells. Galactocerebroside, which contains a glucose or galactose head group, are characteristic of the brain. Other cerebroside are found in nonneural tissues. Gangliosides, whose head groups contain multiple sugar units, are abundant in the brain, but are also found in nonneural tissues.

30 Sphingolipids are built on a sphingosine backbone. Sphingosine is acylated to ceramide by the enzyme sphingosine acetyltransferase. Ceramide and phosphatidyl choline are converted to sphingomyelin by the enzyme ceramide choline phosphotransferase. Cerebroside are synthesized by the linkage of glucose or galactose to ceramide by a transferase. Sequential addition of sugar residues to ceramide by transferase enzymes yields gangliosides.

Eicosanoid Metabolism

Eicosanoids, including prostaglandins, prostacyclin, thromboxanes, and leukotrienes, are 20-carbon molecules derived from fatty acids. Eicosanoids are signaling molecules which have roles in pain, fever, and inflammation. The precursor of all eicosanoids is arachidonate, which is generated from phospholipids by phospholipase A₂ and from diacylglycerols by diacylglycerol lipase. Leukotrienes are produced from arachidonate by the action of lipoxygenases. Prostaglandin synthase, reductases, and isomerases are responsible for the synthesis of the prostaglandins. Prostaglandins have roles in inflammation, blood flow, ion transport, synaptic transmission, and sleep. Prostacyclin and the thromboxanes are derived from a precursor prostaglandin by the action of prostacyclin synthase and thromboxane synthases, respectively.

Ketone Body Metabolism

Pairs of acetyl-CoA molecules derived from fatty acid oxidation in the liver can condense to form acetoacetyl-CoA, which subsequently forms acetoacetate, D-3-hydroxybutyrate, and acetone. These three products are known as ketone bodies. Enzymes involved in ketone body metabolism include HMG-CoA synthetase, HMG-CoA cleavage enzyme, D-3-hydroxybutyrate dehydrogenase, acetoacetate decarboxylase, and 3-ketoacyl-CoA transferase. Ketone bodies are a normal fuel supply of the heart and renal cortex. Acetoacetate produced by the liver is transported to cells where the acetoacetate is converted back to acetyl-CoA and enters the citric acid cycle. In times of starvation, ketone bodies produced from stored triacylglycerols become an important fuel source, especially for the brain. Abnormally high levels of ketone bodies are observed in diabetics. Diabetic coma can result if ketone body levels become too great.

Lipid Mobilization

Within cells, fatty acids are transported by cytoplasmic fatty acid binding proteins (Online Mendelian Inheritance in Man (OMIM) *134650 Fatty Acid-Binding Protein 1, Liver; FABP1). Diazepam binding inhibitor (DBI), also known as endozepine and acyl CoA-binding protein, is an endogenous γ -aminobutyric acid (GABA) receptor ligand which is thought to down-regulate the effects of GABA. DBI binds medium- and long-chain acyl-CoA esters with very high affinity and may function as an intracellular carrier of acyl-CoA esters (OMIM *125950 Diazepam Binding Inhibitor; DBI; PROSITE PDOC00686 Acyl-CoA-binding protein signature).

Fat stored in liver and adipose triglycerides may be released by hydrolysis and transported in the blood. Free fatty acids are transported in the blood by albumin. Triacylglycerols and cholesterol esters in the blood are transported in lipoprotein particles. The particles consist of a core of hydrophobic lipids surrounded by a shell of polar lipids and apolipoproteins. The protein components serve in the solubilization of hydrophobic lipids and also contain cell-targeting signals. Lipoproteins

include chylomicrons, chylomicron remnants, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).

There is a strong inverse correlation between the levels of plasma HDL and risk of premature coronary heart disease.

5 Triacylglycerols in chylomicrons and VLDL are hydrolyzed by lipoprotein lipases that line blood vessels in muscle and other tissues that use fatty acids. Cell surface LDL receptors bind LDL particles which are then internalized by endocytosis. Absence of the LDL receptor, the cause of the disease familial hypercholesterolemia, leads to increased plasma cholesterol levels and ultimately to atherosclerosis. Plasma cholesteryl ester transfer protein mediates the transfer of cholesteryl esters
10 from HDL to apolipoprotein B-containing lipoproteins. Cholesteryl ester transfer protein is important in the reverse cholesterol transport system and may play a role in atherosclerosis (Yamashita, S. et al. (1997) *Curr. Opin. Lipidol.* 8:101-110). Macrophage scavenger receptors, which bind and internalize modified lipoproteins, play a role in lipid transport and may contribute to atherosclerosis (Greaves, D.R. et al. (1998) *Curr. Opin. Lipidol.* 9:425-432).

15 Proteins involved in cholesterol uptake and biosynthesis are tightly regulated in response to cellular cholesterol levels. The sterol regulatory element binding protein (SREBP) is a sterol-responsive transcription factor. Under normal cholesterol conditions, SREBP resides in the ER membrane. When cholesterol levels are low, a regulated cleavage of SREBP occurs which releases the extracellular domain of the protein. This cleaved domain is then transported to the nucleus where it activates the
20 transcription of the LDL receptor gene, and genes encoding enzymes of cholesterol synthesis, by binding the sterol regulatory element (SRE) upstream of the genes (Yang, J. et al. (1995) *J. Biol. Chem.* 270:12152-12161). Regulation of cholesterol uptake and biosynthesis also occurs via the oxysterol-binding protein (OSBP). OSBP is a high-affinity intracellular receptor for a variety of oxysterols that down-regulate cholesterol synthesis and stimulate cholesterol esterification (Lagace, T.A. et al. (1997)
25 *Biochem. J.* 326:205-213).

Beta-oxidation

 Mitochondrial and peroxisomal beta-oxidation enzymes degrade saturated and unsaturated fatty acids by sequential removal of two-carbon units from CoA-activated fatty acids. The main beta-oxidation pathway degrades both saturated and unsaturated fatty acids while the auxiliary pathway
30 performs additional steps required for the degradation of unsaturated fatty acids.

 The pathways of mitochondrial and peroxisomal beta-oxidation use similar enzymes, but have different substrate specificities and functions. Mitochondria oxidize short-, medium-, and long-chain fatty acids to produce energy for cells. Mitochondrial beta-oxidation is a major energy source for cardiac and skeletal muscle. In liver, it provides ketone bodies to the peripheral circulation when

glucose levels are low as in starvation, endurance exercise, and diabetes (Eaton, S. et al. (1996) Biochem. J. 320:345-357). Peroxisomes oxidize medium-, long-, and very-long-chain fatty acids, dicarboxylic fatty acids, branched fatty acids, prostaglandins, xenobiotics, and bile acid intermediates. The chief roles of peroxisomal beta-oxidation are to shorten toxic lipophilic carboxylic acids to
5 facilitate their excretion and to shorten very-long-chain fatty acids prior to mitochondrial beta-oxidation (Mannaerts, G.P. and P.P. van Veldhoven (1993) Biochimie 75:147-158).

Enzymes involved in beta-oxidation include acyl CoA synthetase, carnitine acyltransferase, acyl CoA dehydrogenases, enoyl CoA hydratases, L-3-hydroxyacyl CoA dehydrogenase, β -ketothiolase, 2,4-dienoyl CoA reductase, and isomerase.

10 Lipid Cleavage and Degradation

Triglycerides are hydrolyzed to fatty acids and glycerol by lipases. Lysophospholipases (LPLs) are widely distributed enzymes that metabolize intracellular lipids, and occur in numerous isoforms. Small isoforms, approximately 15-30 kD, function as hydrolases; large isoforms, those exceeding 60 kD, function both as hydrolases and transacylases. A particular substrate for LPLs,
15 lysophosphatidylcholine, causes lysis of cell membranes when it is formed or imported into a cell. LPLs are regulated by lipid factors including acylcarnitine, arachidonic acid, and phosphatidic acid. These lipid factors are signaling molecules important in numerous pathways, including the inflammatory response. (Anderson, R. et al. (1994) Toxicol. Appl. Pharmacol. 125:176-183; Selle, H. et al. (1993); Eur. J. Biochem. 212:411-416.)

20 The secretory phospholipase A₂ (PLA₂) superfamily comprises a number of heterogeneous enzymes whose common feature is to hydrolyze the sn-2 fatty acid acyl ester bond of phosphoglycerides. Hydrolysis of the glycerophospholipids releases free fatty acids and lysophospholipids. PLA₂ activity generates precursors for the biosynthesis of biologically active lipids, hydroxy fatty acids, and platelet-activating factor. PLA₂ hydrolysis of the sn-2 ester bond in
25 phospholipids generates free fatty acids, such as arachidonic acid and lysophospholipids.

Carbon and Carbohydrate Metabolism

Carbohydrates, including sugars or saccharides, starch, and cellulose, are aldehyde or ketone compounds with multiple hydroxyl groups. The importance of carbohydrate metabolism is demonstrated by the sensitive regulatory system in place for maintenance of blood glucose levels. Two
30 pancreatic hormones, insulin and glucagon, promote increased glucose uptake and storage by cells, and increased glucose release from cells, respectively. Carbohydrates have three important roles in mammalian cells. First, carbohydrates are used as energy stores, fuels, and metabolic intermediates. Carbohydrates are broken down to form energy in glycolysis and are stored as glycogen for later use. Second, the sugars deoxyribose and ribose form part of the structural support of DNA and RNA,

respectively. Third, carbohydrate modifications are added to secreted and membrane proteins and lipids as they traverse the secretory pathway. Cell surface carbohydrate-containing macromolecules, including glycoproteins, glycolipids, and transmembrane proteoglycans, mediate adhesion with other cells and with components of the extracellular matrix. The extracellular matrix is comprised of diverse glycoproteins, glycosaminoglycans (GAGs), and carbohydrate-binding proteins which are secreted from the cell and assembled into an organized meshwork in close association with the cell surface. The interaction of the cell with the surrounding matrix profoundly influences cell shape, strength, flexibility, motility, and adhesion. These dynamic properties are intimately associated with signal transduction pathways controlling cell proliferation and differentiation, tissue construction, and embryonic development.

Carbohydrate metabolism is altered in several disorders including diabetes mellitus, hyperglycemia, hypoglycemia, galactosemia, galactokinase deficiency, and UDP-galactose-4-epimerase deficiency (Fauci, A.S. et al. (1998) Harrison's Principles of Internal Medicine, McGraw-Hill, New York NY, pp. 2208-2209). Altered carbohydrate metabolism is associated with cancer. Reduced GAG and proteoglycan expression is associated with human lung carcinomas (Nackaerts, K. et al. (1997) *Int. J. Cancer* 74:335-345). The carbohydrate determinants sialyl Lewis A and sialyl Lewis X are frequently expressed on human cancer cells (Kannagi, R. (1997) *Glycoconj. J.* 14:577-584). Alterations of the N-linked carbohydrate core structure of cell surface glycoproteins are linked to colon and pancreatic cancers (Schwarz, R.E. et al. (1996) *Cancer Lett.* 107:285-291). Reduced expression of the Sda blood group carbohydrate structure in cell surface glycolipids and glycoproteins is observed in gastrointestinal cancer (Dohi, T. et al. (1996) *Int. J. Cancer* 67:626-663). (Carbon and carbohydrate metabolism is reviewed in Stryer, L. (1995) Biochemistry W.H. Freeman and Company, New York NY; Lehninger, A.L. (1982) Principles of Biochemistry Worth Publishers Inc., New York NY; and Lodish, H. et al. (1995) Molecular Cell Biology Scientific American Books, New York NY.)

Glycolysis

Enzymes of the glycolytic pathway convert the sugar glucose to pyruvate while simultaneously producing ATP. The pathway also provides building blocks for the synthesis of cellular components such as long-chain fatty acids. After glycolysis, pyruvate is converted to acetyl-Coenzyme A, which, in aerobic organisms, enters the citric acid cycle. Glycolytic enzymes include hexokinase, phosphoglucose isomerase, phosphofructokinase, aldolase, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase, enolase, and pyruvate kinase. Of these, phosphofructokinase, hexokinase, and pyruvate kinase are important in regulating the rate of glycolysis.

Gluconeogenesis

Gluconeogenesis is the synthesis of glucose from noncarbohydrate precursors such as lactate and amino acids. The pathway, which functions mainly in times of starvation and intense exercise, occurs mostly in the liver and kidney. Responsible enzymes include pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase, and glucose-6-phosphatase.

5 Pentose Phosphate Pathway

Pentose phosphate pathway enzymes are responsible for generating the reducing agent NADPH, while at the same time oxidizing glucose-6-phosphate to ribose-5-phosphate. Ribose-5-phosphate and its derivatives become part of important biological molecules such as ATP, Coenzyme A, NAD⁺, FAD, RNA, and DNA. The pentose phosphate pathway has both oxidative and non-oxidative branches. The oxidative branch steps, which are catalyzed by the enzymes glucose-6-phosphate dehydrogenase, lactonase, and 6-phosphogluconate dehydrogenase, convert glucose-6-phosphate and NADP⁺ to ribulose-6-phosphate and NADPH. The non-oxidative branch steps, which are catalyzed by the enzymes phosphopentose isomerase, phosphopentose epimerase, transketolase, and transaldolase, allow the interconversion of three-, four-, five-, six-, and seven-carbon sugars.

15 Glucuronate Metabolism

Glucuronate is a monosaccharide which, in the form of D-glucuronic acid, is found in the GAGs chondroitin and dermatan. D-glucuronic acid is also important in the detoxification and excretion of foreign organic compounds such as phenol. Enzymes involved in glucuronate metabolism include UDP-glucose dehydrogenase and glucuronate reductase.

20 Disaccharide Metabolism

Disaccharides must be hydrolyzed to monosaccharides to be digested. Lactose, a disaccharide found in milk, is hydrolyzed to galactose and glucose by the enzyme lactase. Maltose is derived from plant starch and is hydrolyzed to glucose by the enzyme maltase. Sucrose is derived from plants and is hydrolyzed to glucose and fructose by the enzyme sucrase. Trehalose, a disaccharide found mainly in insects and mushrooms, is hydrolyzed to glucose by the enzyme trehalase (OMIM *275360 Trehalase; Ruf, J. et al. (1990) J. Biol. Chem. 265:15034-15039). Lactase, maltase, sucrase, and trehalase are bound to mucosal cells lining the small intestine, where they participate in the digestion of dietary disaccharides. The enzyme lactose synthetase, composed of the catalytic subunit galactosyltransferase and the modifier subunit α -lactalbumin, converts UDP-galactose and glucose to lactose in the mammary glands.

30 Glycogen, Starch, and Chitin Metabolism

Glycogen is the storage form of carbohydrates in mammals. Mobilization of glycogen maintains glucose levels between meals and during muscular activity. Glycogen is stored mainly in the liver and in skeletal muscle in the form of cytoplasmic granules. These granules contain enzymes that

catalyze the synthesis and degradation of glycogen, as well as enzymes that regulate these processes. Enzymes that catalyze the degradation of glycogen include glycogen phosphorylase, a transferase, α -1,6-glucosidase, and phosphoglucomutase. Enzymes that catalyze the synthesis of glycogen include UDP-glucose pyrophosphorylase, glycogen synthetase, a branching enzyme, and nucleoside diphosphokinase. The enzymes of glycogen synthesis and degradation are tightly regulated by the hormones insulin, glucagon, and epinephrine. Starch, a plant-derived polysaccharide, is hydrolyzed to maltose, maltotriose, and α -dextrin by α -amylase, an enzyme secreted by the salivary glands and pancreas. Chitin is a polysaccharide found in insects and crustacea. A chitotriosidase is secreted by macrophages and may play a role in the degradation of chitin-containing pathogens (Boot, R.G. et al. (1995) J. Biol. Chem. 270:26252-26256).

Peptidoglycans and Glycosaminoglycans

Glycosaminoglycans (GAGs) are anionic linear unbranched polysaccharides composed of repetitive disaccharide units. These repetitive units contain a derivative of an amino sugar, either glucosamine or galactosamine. GAGs exist free or as part of proteoglycans, large molecules composed of a core protein attached to one or more GAGs. GAGs are found on the cell surface, inside cells, and in the extracellular matrix. Changes in GAG levels are associated with several autoimmune diseases including autoimmune thyroid disease, autoimmune diabetes mellitus, and systemic lupus erythematosus (Hansen, C. et al. (1996) Clin. Exp. Rheum. 14 (Suppl. 15):S59-S67). GAGs include chondroitin sulfate, keratan sulfate, heparin, heparan sulfate, dermatan sulfate, and hyaluronan.

The GAG hyaluronan (HA) is found in the extracellular matrix of many cells, especially in soft connective tissues, and is abundant in synovial fluid (Pitsillides, A.A. et al. (1993) Int. J. Exp. Pathol. 74:27-34). HA seems to play important roles in cell regulation, development, and differentiation (Laurent, T.C. and J.R. Fraser (1992) FASEB J. 6:2397-2404). Hyaluronidase is an enzyme that degrades HA to oligosaccharides. Hyaluronidases may function in cell adhesion, infection, angiogenesis, signal transduction, reproduction, cancer, and inflammation.

Proteoglycans, also known as peptidoglycans, are found in the extracellular matrix of connective tissues such as cartilage and are essential for distributing the load in weight-bearing joints. Cell-surface-attached proteoglycans anchor cells to the extracellular matrix. Both extracellular and cell-surface proteoglycans bind growth factors, facilitating their binding to cell-surface receptors and subsequent triggering of signal transduction pathways.

Amino Acid and Nitrogen Metabolism

NH_4^+ is assimilated into amino acids by the actions of two enzymes, glutamate dehydrogenase and glutamine synthetase. The carbon skeletons of amino acids come from the intermediates of glycolysis, the pentose phosphate pathway, or the citric acid cycle. Of the twenty

amino acids used in proteins, humans can synthesize only thirteen (nonessential amino acids). The remaining nine must come from the diet (essential amino acids). Enzymes involved in nonessential amino acid biosynthesis include glutamate kinase dehydrogenase, pyrroline carboxylate reductase, asparagine synthetase, phenylalanine oxygenase, methionine adenosyltransferase, adenosylhomocysteinase, cystathionine β -synthase, cystathionine γ -lyase, phosphoglycerate dehydrogenase, phosphoserine transaminase, phosphoserine phosphatase, serine hydroxymethyltransferase, and glycine synthase.

Metabolism of amino acids takes place almost entirely in the liver, where the amino group is removed by aminotransferases (transaminases), for example, alanine aminotransferase. The amino group is transferred to α -ketoglutarate to form glutamate. Glutamate dehydrogenase converts glutamate to NH_4^+ and α -ketoglutarate. NH_4^+ is converted to urea by the urea cycle which is catalyzed by the enzymes arginase, ornithine transcarbamoylase, arginosuccinate synthetase, and arginosuccinase. Carbamoyl phosphate synthetase is also involved in urea formation. Enzymes involved in the metabolism of the carbon skeleton of amino acids include serine dehydratase, asparaginase, glutaminase, propionyl CoA carboxylase, methylmalonyl CoA mutase, branched-chain α -keto dehydrogenase complex, isovaleryl CoA dehydrogenase, β -methylcrotonyl CoA carboxylase, phenylalanine hydroxylase, p-hydroxylphenylpyruvate hydroxylase, and homogentisate oxidase.

Polyamines, which include spermidine, putrescine, and spermine, bind tightly to nucleic acids and are abundant in rapidly proliferating cells. Enzymes involved in polyamine synthesis include ornithine decarboxylase.

Diseases involved in amino acid and nitrogen metabolism include hyperammonemia, carbamoyl phosphate synthetase deficiency, urea cycle enzyme deficiencies, methylmalonic aciduria, maple syrup disease, alcaptonuria, and phenylketonuria.

Energy Metabolism

Cells derive energy from metabolism of ingested compounds that may be roughly categorized as carbohydrates, fats, or proteins. Energy is also stored in polymers such as triglycerides (fats) and glycogen (carbohydrates). Metabolism proceeds along separate reaction pathways connected by key intermediates such as acetyl coenzyme A (acetyl-CoA). Metabolic pathways feature anaerobic and aerobic degradation, coupled with the energy-requiring reactions such as phosphorylation of adenosine diphosphate (ADP) to the triphosphate (ATP) or analogous phosphorylations of guanosine (GDP/GTP), uridine (UDP/UTP), or cytidine (CDP/CTP). Subsequent dephosphorylation of the triphosphate drives reactions needed for cell maintenance, growth, and proliferation.

Digestive enzymes convert carbohydrates and sugars to glucose; fructose and galactose are converted in the liver to glucose. Enzymes involved in these conversions include galactose-1-

phosphate uridyl transferase and UDP-galactose-4 epimerase. In the cytoplasm, glycolysis converts glucose to pyruvate in a series of reactions coupled to ATP synthesis.

Pyruvate is transported into the mitochondria and converted to acetyl-CoA for oxidation via the citric acid cycle, involving pyruvate dehydrogenase components, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase. Enzymes involved in the citric acid cycle include: citrate synthetase, aconitases, isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase complex including transsuccinylases, succinyl CoA synthetase, succinate dehydrogenase, fumarases, and malate dehydrogenase. Acetyl CoA is oxidized to CO₂ with concomitant formation of NADH, FADH₂, and GTP. In oxidative phosphorylation, the transport of electrons from NADH and FADH₂ to oxygen by dehydrogenases is coupled to the synthesis of ATP from ADP and P_i by the F₀F₁ ATPase complex in the mitochondrial inner membrane. Enzyme complexes responsible for electron transport and ATP synthesis include the F₀F₁ ATPase complex, ubiquinone(CoQ)-cytochrome c reductase, ubiquinone reductase, cytochrome b, cytochrome c₁, FeS protein, and cytochrome c oxidase.

Triglycerides are hydrolyzed to fatty acids and glycerol by lipases. Glycerol is then phosphorylated to glycerol-3-phosphate by glycerol kinase and glycerol phosphate dehydrogenase, and degraded by the glycolysis. Fatty acids are transported into the mitochondria as fatty acyl-carnitine esters and undergo oxidative degradation.

In addition to metabolic disorders such as diabetes and obesity, disorders of energy metabolism are associated with cancers (Dorward, A. et al. (1997) J. Bioenerg. Biomembr. 29:385-392), autism (Lombard, J. (1998) Med. Hypotheses 50:497-500), neurodegenerative disorders (Alexi, T. et al. (1998) Neuroreport 9:R57-64), and neuromuscular disorders (DiMauro, S. et al. (1998) Biochim. Biophys. Acta 1366:199-210). The myocardium is heavily dependent on oxidative metabolism, so metabolic dysfunction often leads to heart disease (DiMauro, S. and M. Hirano (1998) Curr. Opin. Cardiol. 13:190-197).

For a review of energy metabolism enzymes and intermediates, see Stryer, L. et al. (1995) Biochemistry, W.H. Freeman and Co., San Francisco CA, pp. 443-652. For a review of energy metabolism regulation, see Lodish, H. et al. (1995) Molecular Cell Biology, Scientific American Books, New York NY, pp. 744-770.

Cofactor Metabolism

Cofactors, including coenzymes and prosthetic groups, are small molecular weight inorganic or organic compounds that are required for the action of an enzyme. Many cofactors contain vitamins as a component. Cofactors include thiamine pyrophosphate, flavin adenine dinucleotide, flavin mononucleotide, nicotinamide adenine dinucleotide, pyridoxal phosphate, coenzyme A, tetrahydrofolate, lipoamide, and heme. The vitamins biotin and cobalamin are associated with

enzymes as well. Heme, a prosthetic group found in myoglobin and hemoglobin, consists of protoporphyrin group bound to iron. Porphyrin groups contain four substituted pyrroles covalently joined in a ring, often with a bound metal atom. Enzymes involved in porphyrin synthesis include δ -aminolevulinate synthase, δ -aminolevulinate dehydrase, porphobilinogen deaminase, and cosynthase.

5 Deficiencies in heme formation cause porphyrias. Heme is broken down as a part of erythrocyte turnover. Enzymes involved in heme degradation include heme oxygenase and biliverdin reductase.

Iron is a required cofactor for many enzymes. Besides the heme-containing enzymes, iron is found in iron-sulfur clusters in proteins including aconitase, succinate dehydrogenase, and NADH-Q reductase. Iron is transported in the blood by the protein transferrin. Binding of transferrin to the
10 transferrin receptor on cell surfaces allows uptake by receptor mediated endocytosis. Cytosolic iron is bound to ferritin protein.

A molybdenum-containing cofactor (molybdopterin) is found in enzymes including sulfite oxidase, xanthine dehydrogenase, and aldehyde oxidase. Molybdopterin biosynthesis is performed by two molybdenum cofactor synthesizing enzymes. Deficiencies in these enzymes cause mental
15 retardation and lens dislocation. Other diseases caused by defects in cofactor metabolism include pernicious anemia and methylmalonic aciduria.

Secretion and Trafficking

Eukaryotic cells are bound by a lipid bilayer membrane and subdivided into functionally distinct, membrane bound compartments. The membranes maintain the essential differences between
20 the cytosol, the extracellular environment, and the luminal space of each intracellular organelle. As lipid membranes are highly impermeable to most polar molecules, transport of essential nutrients, metabolic waste products, cell signaling molecules, macromolecules and proteins across lipid membranes and between organelles must be mediated by a variety of transport-associated molecules.

Protein Trafficking

25 In eukaryotes, some proteins are synthesized on ER-bound ribosomes, co-translationally imported into the ER, delivered from the ER to the Golgi complex for post-translational processing and sorting, and transported from the Golgi to specific intracellular and extracellular destinations. All cells possess a constitutive transport process which maintains homeostasis between the cell and its environment. In many differentiated cell types, the basic machinery is modified to carry out specific
30 transport functions. For example, in endocrine glands, hormones and other secreted proteins are packaged into secretory granules for regulated exocytosis to the cell exterior. In macrophage, foreign extracellular material is engulfed (phagocytosis) and delivered to lysosomes for degradation. In fat and muscle cells, glucose transporters are stored in vesicles which fuse with the plasma membrane only in response to insulin stimulation.

The Secretory Pathway

Synthesis of most integral membrane proteins, secreted proteins, and proteins destined for the lumen of a particular organelle occurs on ER-bound ribosomes. These proteins are co-translationally imported into the ER. The proteins leave the ER via membrane-bound vesicles which bud off the ER at specific sites and fuse with each other (homotypic fusion) to form the ER-Golgi Intermediate Compartment (ERGIC). The ERGIC matures progressively through the *cis*, *medial*, and *trans* cisternal stacks of the Golgi, modifying the enzyme composition by retrograde transport of specific Golgi enzymes. In this way, proteins moving through the Golgi undergo post-translational modification, such as glycosylation. The final Golgi compartment is the Trans-Golgi Network (TGN), where both membrane and luminal proteins are sorted for their final destination. Transport vesicles destined for intracellular compartments, such as the lysosome, bud off the TGN. What remains is a secretory vesicle which contains proteins destined for the plasma membrane, such as receptors, adhesion molecules, and ion channels, and secretory proteins, such as hormones, neurotransmitters, and digestive enzymes. Secretory vesicles eventually fuse with the plasma membrane (Glick, B.S. and V. Malhotra (1998) Cell 95:883-889).

The secretory process can be constitutive or regulated. Most cells have a constitutive pathway for secretion, whereby vesicles derived from maturation of the TGN require no specific signal to fuse with the plasma membrane. In many cells, such as endocrine cells, digestive cells, and neurons, vesicle pools derived from the TGN collect in the cytoplasm and do not fuse with the plasma membrane until they are directed to by a specific signal.

Endocytosis

Endocytosis, wherein cells internalize material from the extracellular environment, is essential for transmission of neuronal, metabolic, and proliferative signals; uptake of many essential nutrients; and defense against invading organisms. Most cells exhibit two forms of endocytosis. The first, phagocytosis, is an actin-driven process exemplified in macrophage and neutrophils. Material to be endocytosed contacts numerous cell surface receptors which stimulate the plasma membrane to extend and surround the particle, enclosing it in a membrane-bound phagosome. In the mammalian immune system, IgG-coated particles bind Fc receptors on the surface of phagocytic leukocytes. Activation of the Fc receptors initiates a signal cascade involving src-family cytosolic kinases and the monomeric GTP-binding (G) protein Rho. The resulting actin reorganization leads to phagocytosis of the particle. This process is an important component of the humoral immune response, allowing the processing and presentation of bacterial-derived peptides to antigen-specific T-lymphocytes.

The second form of endocytosis, pinocytosis, is a more generalized uptake of material from the external milieu. Like phagocytosis, pinocytosis is activated by ligand binding to cell surface receptors.

Activation of individual receptors stimulates an internal response that includes coalescence of the receptor-ligand complexes and formation of clathrin-coated pits. Invagination of the plasma membrane at clathrin-coated pits produces an endocytic vesicle within the cell cytoplasm. These vesicles undergo homotypic fusion to form an early endosomal (EE) compartment. The tubulovesicular EE serves as a sorting site for incoming material. ATP-driven proton pumps in the EE membrane lowers the pH of the EE lumen (pH 6.3-6.8). The acidic environment causes many ligands to dissociate from their receptors. The receptors, along with membrane and other integral membrane proteins, are recycled back to the plasma membrane by budding off the tubular extensions of the EE in recycling vesicles (RV). This selective removal of recycled components produces a carrier vesicle containing ligand and other material from the external environment. The carrier vesicle fuses with TGN-derived vesicles which contain hydrolytic enzymes. The acidic environment of the resulting late endosome (LE) activates the hydrolytic enzymes which degrade the ligands and other material. As digestion takes place, the LE fuses with the lysosome where digestion is completed (Mellman, I. (1996) *Annu. Rev. Cell Dev. Biol.* 12:575-625).

Recycling vesicles may return directly to the plasma membrane. Receptors internalized and returned directly to the plasma membrane have a turnover rate of 2-3 minutes. Some RVs undergo microtubule-directed relocation to a perinuclear site, from which they then return to the plasma membrane. Receptors following this route have a turnover rate of 5-10 minutes. Still other RVs are retained within the cell until an appropriate signal is received (Mellman, *supra*; and James, D.E. et al. (1994) *Trends Cell Biol.* 4:120-126).

Vesicle Formation

Several steps in the transit of material along the secretory and endocytic pathways require the formation of transport vesicles. Specifically, vesicles form at the transitional endoplasmic reticulum (tER), the rim of Golgi cisternae, the face of the Trans-Golgi Network (TGN), the plasma membrane (PM), and tubular extensions of the endosomes. The process begins with the budding of a vesicle out of the donor membrane. The membrane-bound vesicle contains proteins to be transported and is surrounded by a protective coat made up of protein subunits recruited from the cytosol. The initial budding and coating processes are controlled by a cytosolic ras-like GTP-binding protein, ADP-ribosylating factor (Arf), and adapter proteins (AP). Different isoforms of both Arf and AP are involved at different sites of budding. Another small G-protein, dynamin, forms a ring complex around the neck of the forming vesicle and may provide the mechanochemical force to accomplish the final step of the budding process. The coated vesicle complex is then transported through the cytosol. During the transport process, Arf-bound GTP is hydrolyzed to GDP and the coat dissociates from the transport vesicle (West, M.A. et al. (1997) *J. Cell Biol.* 138:1239-1254). Two different classes of coat protein

have also been identified. Clathrin coats form on the TGN and PM surfaces, whereas coatomer or COP coats form on the ER and Golgi. COP coats can further be distinguished as COPI, involved in retrograde traffic through the Golgi and from the Golgi to the ER, and COPII, involved in anterograde traffic from the ER to the Golgi (Mellman, supra). The COP coat consists of two major components, a
5 G-protein (Arf or Sar) and coat protomer (coatomer). Coatomer is an equimolar complex of seven proteins, termed alpha-, beta-, beta'-, gamma-, delta-, epsilon- and zeta-COP. (Harter, C. and F.T. Wieland (1998) Proc. Natl. Acad. Sci. USA 95:11649-11654.)

Membrane Fusion

Transport vesicles undergo homotypic or heterotypic fusion in the secretory and endocytotic
10 pathways. Molecules required for appropriate targeting and fusion of vesicles with their target membrane include proteins incorporated in the vesicle membrane, the target membrane, and proteins recruited from the cytosol. During budding of the vesicle from the donor compartment, an integral membrane protein, VAMP (vesicle-associated membrane protein) is incorporated into the vesicle. Soon after the vesicle uncoats, a cytosolic prenylated GTP-binding protein, Rab (a member of the Ras
15 superfamily), is inserted into the vesicle membrane. GTP-bound Rab proteins are directed into nascent transport vesicles where they interact with VAMP. Following vesicle transport, GTPase activating proteins (GAPs) in the target membrane convert Rab proteins to the GDP-bound form. A cytosolic protein, guanine-nucleotide dissociation inhibitor (GDI) helps return GDP-bound Rab proteins to their
20 membrane of origin. Several Rab isoforms have been identified and appear to associate with specific compartments within the cell. Rab proteins appear to play a role in mediating the function of a viral gene, Rev, which is essential for replication of HIV-1, the virus responsible for AIDS (Flavell, R.A. et al. (1996) Proc. Natl. Acad. Sci. USA 93:4421-4424).

Docking of the transport vesicle with the target membrane involves the formation of a complex between the vesicle SNAP receptor (v-SNARE), target membrane (t-) SNAREs, and certain other
25 membrane and cytosolic proteins. Many of these other proteins have been identified although their exact functions in the docking complex remain uncertain (Tellam, J.T. et al. (1995) J. Biol. Chem. 270:5857-5863; and Hata, Y. and T.C. Sudhof (1995) J. Biol. Chem. 270:13022-13028). N-ethylmaleimide sensitive factor (NSF) and soluble NSF-attachment protein (α -SNAP and β -SNAP) are two such proteins that are conserved from yeast to man and function in most intracellular membrane
30 fusion reactions. Sec1 represents a family of yeast proteins that function at many different stages in the secretory pathway including membrane fusion. Recently, mammalian homologs of Sec1, called Munc-18 proteins, have been identified (Katagiri, H. et al. (1995) J. Biol. Chem. 270:4963-4966; Hata et al. supra).

The SNARE complex involves three SNARE molecules, one in the vesicular membrane and

two in the target membrane. Synaptotagmin is an integral membrane protein in the synaptic vesicle which associates with the t-SNARE syntaxin in the docking complex. Synaptotagmin binds calcium in a complex with negatively charged phospholipids, which allows the cytosolic SNAP protein to displace synaptotagmin from syntaxin and fusion to occur. Thus, synaptotagmin is a negative regulator of fusion in the neuron (Littleton, J.T. et al. (1993) Cell 74:1125-1134). The most abundant membrane protein of synaptic vesicles appears to be the glycoprotein synaptophysin, a 38 kDa protein with four transmembrane domains.

Specificity between a vesicle and its target is derived from the v-SNARE, t-SNAREs, and associated proteins involved. Different isoforms of SNAREs and Rabs show distinct cellular and subcellular distributions. VAMP-1/synaptobrevin, membrane-anchored synaptosome-associated protein of 25 kDa (SNAP-25), syntaxin-1, Rab3A, Rab15, and Rab23 are predominantly expressed in the brain and nervous system. Different syntaxin, VAMP, and Rab proteins are associated with distinct subcellular compartments and their vesicular carriers.

Nuclear Transport

Transport of proteins and RNA between the nucleus and the cytoplasm occurs through nuclear pore complexes (NPCs). NPC-mediated transport occurs in both directions through the nuclear envelope. All nuclear proteins are imported from the cytoplasm, their site of synthesis. tRNA and mRNA are exported from the nucleus, their site of synthesis, to the cytoplasm, their site of function. Processing of small nuclear RNAs involves export into the cytoplasm, assembly with proteins and modifications such as hypermethylation to produce small nuclear ribonuclear proteins (snRNPs), and subsequent import of the snRNPs back into the nucleus. The assembly of ribosomes requires the initial import of ribosomal proteins from the cytoplasm, their incorporation with RNA into ribosomal subunits, and export back to the cytoplasm. (Görllich, D. and I.W. Mattaj (1996) Science 271:1513-1518.)

The transport of proteins and mRNAs across the NPC is selective, dependent on nuclear localization signals, and generally requires association with nuclear transport factors. Nuclear localization signals (NLS) consist of short stretches of amino acids enriched in basic residues. NLS are found on proteins that are targeted to the nucleus, such as the glucocorticoid receptor. The NLS is recognized by the NLS receptor, importin, which then interacts with the monomeric GTP-binding protein Ran. This NLS protein/receptor/Ran complex navigates the nuclear pore with the help of the homodimeric protein nuclear transport factor 2 (NTF2). NTF2 binds the GDP-bound form of Ran and to multiple proteins of the nuclear pore complex containing FXFG repeat motifs, such as p62. (Paschal, B. et al. (1997) J. Biol. Chem. 272:21534-21539; and Wong, D.H. et al. (1997) Mol. Cell Biol. 17:3755-3767). Some proteins are dissociated before nuclear mRNAs are transported across the

NPC while others are dissociated shortly after nuclear mRNA transport across the NPC and are reimported into the nucleus.

Disease Correlation

The etiology of numerous human diseases and disorders can be attributed to defects in the transport or secretion of proteins. For example, abnormal hormonal secretion is linked to disorders such as diabetes insipidus (vasopressin), hyper- and hypoglycemia (insulin, glucagon), Grave's disease and goiter (thyroid hormone), and Cushing's and Addison's diseases (adrenocorticotrophic hormone, ACTH). Moreover, cancer cells secrete excessive amounts of hormones or other biologically active peptides. Disorders related to excessive secretion of biologically active peptides by tumor cells include fasting hypoglycemia due to increased insulin secretion from insulinoma-islet cell tumors; hypertension due to increased epinephrine and norepinephrine secreted from pheochromocytomas of the adrenal medulla and sympathetic paraganglia; and carcinoid syndrome, which is characterized by abdominal cramps, diarrhea, and valvular heart disease caused by excessive amounts of vasoactive substances such as serotonin, bradykinin, histamine, prostaglandins, and polypeptide hormones, secreted from intestinal tumors. Biologically active peptides that are ectopically synthesized in and secreted from tumor cells include ACTH and vasopressin (lung and pancreatic cancers); parathyroid hormone (lung and bladder cancers); calcitonin (lung and breast cancers); and thyroid-stimulating hormone (medullary thyroid carcinoma). Such peptides may be useful as diagnostic markers for tumorigenesis (Schwartz, M.Z. (1997) Semin. Pediatr. Surg. 3:141-146; and Said, S.I. and G.R. Faloona (1975) N. Engl. J. Med. 293:155-160).

Defective nuclear transport may play a role in cancer. The BRCA1 protein contains three potential NLSs which interact with importin alpha, and is transported into the nucleus by the importin/NPC pathway. In breast cancer cells the BRCA1 protein is aberrantly localized in the cytoplasm. The mislocation of the BRCA1 protein in breast cancer cells may be due to a defect in the NPC nuclear import pathway (Chen, C.F. et al. (1996) J. Biol. Chem. 271:32863-32868).

It has been suggested that in some breast cancers, the tumor-suppressing activity of p53 is inactivated by the sequestration of the protein in the cytoplasm, away from its site of action in the cell nucleus. Cytoplasmic wild-type p53 was also found in human cervical carcinoma cell lines. (Moll, U.M. et al. (1992) Proc. Natl. Acad. Sci. USA 89:7262-7266; and Liang, X.H. et al. (1993) Oncogene 8:2645-2652.)

Environmental Responses

Organisms respond to the environment by a number of pathways. Heat shock proteins, including hsp 70, hsp60, hsp90, and hsp 40, assist organisms in coping with heat damage to cellular proteins.

Aquaporins (AQP) are channels that transport water and, in some cases, nonionic small solutes such as urea and glycerol. Water movement is important for a number of physiological processes including renal fluid filtration, aqueous humor generation in the eye, cerebrospinal fluid production in the brain, and appropriate hydration of the lung. Aquaporins are members of the major intrinsic protein (MIP) family of membrane transporters (King, L.S. and P. Agre (1996) *Annu. Rev. Physiol.* 58:619-648; Ishibashi, K. et al. (1997) *J. Biol. Chem.* 272:20782-20786). The study of aquaporins may have relevance to understanding edema formation and fluid balance in both normal physiology and disease states (King, *supra*). Mutations in AQP2 cause autosomal recessive nephrogenic diabetes insipidus (OMIM *107777 Aquaporin 2; AQP2). Reduced AQP4 expression in skeletal muscle may be associated with Duchenne muscular dystrophy (Frigeri, A. et al. (1998) *J. Clin. Invest.* 102:695-703). Mutations in AQP0 cause autosomal dominant cataracts in the mouse (OMIM *154050 Major Intrinsic Protein of Lens Fiber; MIP).

The metallothioneins (MTs) are a group of small (61 amino acids), cysteine-rich proteins that bind heavy metals such as cadmium, zinc, mercury, lead, and copper and are thought to play a role in metal detoxification or the metabolism and homeostasis of metals. Arsenite-resistance proteins have been identified in hamsters that are resistant to toxic levels of arsenite (Rossman, T.G. et al. (1997) *Mutat. Res.* 386:307-314).

Humans respond to light and odors by specific protein pathways. Proteins involved in light perception include rhodopsin, transducin, and cGMP phosphodiesterase. Proteins involved in odor perception include multiple olfactory receptors. Other proteins are important in human Circadian rhythms and responses to wounds.

Immunity and Host Defense

All vertebrates have developed sophisticated and complex immune systems that provide protection from viral, bacterial, fungal and parasitic infections. Included in these systems are the processes of humoral immunity, the complement cascade and the inflammatory response (Paul, W.E. (1993) *Fundamental Immunology*, Raven Press, Ltd., New York NY, pp.1-20).

The cellular components of the humoral immune system include six different types of leukocytes: monocytes, lymphocytes, polymorphonuclear granulocytes (consisting of neutrophils, eosinophils, and basophils) and plasma cells. Additionally, fragments of megakaryocytes, a seventh type of white blood cell in the bone marrow, occur in large numbers in the blood as platelets.

Leukocytes are formed from two stem cell lineages in bone marrow. The myeloid stem cell line produces granulocytes and monocytes and, the lymphoid stem cell produces lymphocytes. Lymphoid cells travel to the thymus, spleen and lymph nodes, where they mature and differentiate into lymphocytes. Leukocytes are responsible for defending the body against invading pathogens.

Neutrophils and monocytes attack invading bacteria, viruses, and other pathogens and destroy them by phagocytosis. Monocytes enter tissues and differentiate into macrophages which are extremely phagocytic. Lymphocytes and plasma cells are a part of the immune system which recognizes specific foreign molecules and organisms and inactivates them, as well as signals other cells to attack the invaders.

Granulocytes and monocytes are formed and stored in the bone marrow until needed. Megakaryocytes are produced in bone marrow, where they fragment into platelets and are released into the bloodstream. The main function of platelets is to activate the blood clotting mechanism. Lymphocytes and plasma cells are produced in various lymphogenous organs, including the lymph nodes, spleen, thymus, and tonsils.

Both neutrophils and macrophages exhibit chemotaxis towards sites of inflammation. Tissue inflammation in response to pathogen invasion results in production of chemo-attractants for leukocytes, such as endotoxins or other bacterial products, prostaglandins, and products of leukocytes or platelets.

Basophils participate in the release of the chemicals involved in the inflammatory process. The main function of basophils is secretion of these chemicals to such a degree that they have been referred to as "unicellular endocrine glands." A distinct aspect of basophilic secretion is that the contents of granules go directly into the extracellular environment, not into vacuoles as occurs with neutrophils, eosinophils and monocytes. Basophils have receptors for the Fc fragment of immunoglobulin E (IgE) that are not present on other leukocytes. Crosslinking of membrane IgE with anti-IgE or other ligands triggers degranulation.

Eosinophils are bi- or multi-nucleated white blood cells which contain eosinophilic granules. Their plasma membrane is characterized by Ig receptors, particularly IgG and IgE. Generally, eosinophils are stored in the bone marrow until recruited for use at a site of inflammation or invasion. They have specific functions in parasitic infections and allergic reactions, and are thought to detoxify some of the substances released by mast cells and basophils which cause inflammation. Additionally, they phagocytize antigen-antibody complexes and further help prevent spread of the inflammation.

Macrophages are monocytes that have left the blood stream to settle in tissue. Once monocytes have migrated into tissues, they do not re-enter the bloodstream. The mononuclear phagocyte system is comprised of precursor cells in the bone marrow, monocytes in circulation, and macrophages in tissues. The system is capable of very fast and extensive phagocytosis. A macrophage may phagocytize over 100 bacteria, digest them and extrude residues, and then survive for many more months. Macrophages are also capable of ingesting large particles, including red blood cells and malarial parasites. They increase several-fold in size and transform into macrophages

that are characteristic of the tissue they have entered, surviving in tissues for several months.

Mononuclear phagocytes are essential in defending the body against invasion by foreign pathogens, particularly intracellular microorganisms such as M. tuberculosis, listeria, leishmania and toxoplasma. Macrophages can also control the growth of tumorous cells, via both phagocytosis and
5 secretion of hydrolytic enzymes. Another important function of macrophages is that of processing antigen and presenting them in a biochemically modified form to lymphocytes.

The immune system responds to invading microorganisms in two major ways: antibody production and cell mediated responses. Antibodies are immunoglobulin proteins produced by B-lymphocytes which bind to specific antigens and cause inactivation or promote destruction of the
10 antigen by other cells. Cell-mediated immune responses involve T-lymphocytes (T cells) that react with foreign antigen on the surface of infected host cells. Depending on the type of T cell, the infected cell is either killed or signals are secreted which activate macrophages and other cells to destroy the infected cell (Paul, supra).

T-lymphocytes originate in the bone marrow or liver in fetuses. Precursor cells migrate via
15 the blood to the thymus, where they are processed to mature into T-lymphocytes. This processing is crucial because of positive and negative selection of T cells that will react with foreign antigen and not with self molecules. After processing, T cells continuously circulate in the blood and secondary lymphoid tissues, such as lymph nodes, spleen, certain epithelium-associated tissues in the gastrointestinal tract, respiratory tract and skin. When T-lymphocytes are presented with the
20 complementary antigen, they are stimulated to proliferate and release large numbers of activated T cells into the lymph system and the blood system. These activated T cells can survive and circulate for several days. At the same time, T memory cells are created, which remain in the lymphoid tissue for months or years. Upon subsequent exposure to that specific antigen, these memory cells will respond more rapidly and with a stronger response than induced by the original antigen. This creates
25 an "immunological memory" that can provide immunity for years.

There are two major types of T cells: cytotoxic T cells destroy infected host cells, and helper T cells activate other white blood cells via chemical signals. One class of helper cell, T_H1 , activates macrophages to destroy ingested microorganisms, while another, T_H2 , stimulates the production of antibodies by B cells.

30 Cytotoxic T cells directly attack the infected target cell. In virus-infected cells, peptides derived from viral proteins are generated by the proteasome. These peptides are transported into the ER by the transporter associated with antigen processing (TAP) (Pamer, E. and P. Cresswell (1998) Annu. Rev. Immunol. 16:323-358). Once inside the ER, the peptides bind MHC I chains, and the peptide/MHC I complex is transported to the cell surface. Receptors on the surface of T cells bind to

antigen presented on cell surface MHC molecules. Once activated by binding to antigen, T cells secrete γ -interferon, a signal molecule that induces the expression of genes necessary for presenting viral (or other) antigens to cytotoxic T cells. Cytotoxic T cells kill the infected cell by stimulating programmed cell death.

5 Helper T cells constitute up to 75% of the total T cell population. They regulate the immune functions by producing a variety of lymphokines that act on other cells in the immune system and on bone marrow. Among these lymphokines are: interleukins-2,3,4,5,6; granulocyte-monocyte colony stimulating factor, and γ -interferon.

10 Helper T cells are required for most B cells to respond to antigen. When an activated helper cell contacts a B cell, its centrosome and Golgi apparatus become oriented toward the B cell, aiding the directing of signal molecules, such as transmembrane-bound protein called CD40 ligand, onto the B cell surface to interact with the CD40 transmembrane protein. Secreted signals also help B cells to proliferate and mature and, in some cases, to switch the class of antibody being produced.

15 B-lymphocytes (B cells) produce antibodies which react with specific antigenic proteins presented by pathogens. Once activated, B cells become filled with extensive rough endoplasmic reticulum and are known as plasma cells. As with T cells, interaction of B cells with antigen stimulates proliferation of only those B cells which produce antibody specific to that antigen. There are five classes of antibodies, known as immunoglobulins, which together comprise about 20% of total plasma protein. Each class mediates a characteristic biological response after antigen binding.
20 Upon activation by specific antigen B cells switch from making membrane-bound antibody to secretion of that antibody.

 Antibodies, or immunoglobulins (Ig), are the founding members of the Ig superfamily and the central components of the humoral immune response. Antibodies are either expressed on the surface of B cells or secreted by B cells into the circulation. Antibodies bind and neutralize blood-borne
25 foreign antigens. The prototypical antibody is a tetramer consisting of two identical heavy polypeptide chains (H-chains) and two identical light polypeptide chains (L-chains) interlinked by disulfide bonds. This arrangement confers the characteristic Y-shape to antibody molecules. Antibodies are classified based on their H-chain composition. The five antibody classes, IgA, IgD, IgE, IgG and IgM, are defined by the α , δ , ϵ , γ , and μ H-chain types. There are two types of L-
30 chains, κ and λ , either of which may associate as a pair with any H-chain pair. IgG, the most common class of antibody found in the circulation, is tetrameric, while the other classes of antibodies are generally variants or multimers of this basic structure.

 H-chains and L-chains each contain an N-terminal variable region and a C-terminal constant region. Both H-chains and L-chains contain repeated Ig domains. For example, a typical H-chain

contains four Ig domains, three of which occur within the constant region and one of which occurs within the variable region and contributes to the formation of the antigen recognition site. Likewise, a typical L-chain contains two Ig domains, one of which occurs within the constant region and one of which occurs within the variable region. In addition, H chains such as μ have been shown to
5 associate with other polypeptides during differentiation of the B cell.

Antibodies can be described in terms of their two main functional domains. Antigen recognition is mediated by the Fab (antigen binding fragment) region of the antibody, while effector functions are mediated by the Fc (crystallizable fragment) region. Binding of antibody to an antigen, such as a bacterium, triggers the destruction of the antigen by phagocytic white blood cells such as
10 macrophages and neutrophils. These cells express surface receptors that specifically bind to the antibody Fc region and allow the phagocytic cells to engulf, ingest, and degrade the antibody-bound antigen. The Fc receptors expressed by phagocytic cells are single-pass transmembrane glycoproteins of about 300 to 400 amino acids (Sears, D.W. et al. (1990) J. Immunol. 144:371-378). The extracellular portion of the Fc receptor typically contains two or three Ig domains.

15 Diseases which cause over- or under-abundance of any one type of leukocyte usually result in the entire immune defense system becoming involved. A well-known autoimmune disease is AIDS (Acquired Immunodeficiency Syndrome) where the number of helper T cells is depleted, leaving the patient susceptible to infection by microorganisms and parasites. Another widespread medical condition attributable to the immune system is that of allergic reactions to certain antigens. Allergic
20 reactions include: hay fever, asthma, anaphylaxis, and urticaria (hives). Leukemias are an excess production of white blood cells, to the point where a major portion of the body's metabolic resources are directed solely at proliferation of white blood cells, leaving other tissues to starve. Leukopenia or agranulocytosis occurs when the bone marrow stops producing white blood cells. This leaves the body unprotected against foreign microorganisms, including those which normally inhabit skin,
25 mucous membranes, and gastrointestinal tract. If all white blood cell production stops completely, infection will occur within two days and death may follow only 1 to 4 days later.

Impaired phagocytosis occurs in several diseases, including monocytic leukemia, systemic lupus, and granulomatous disease. In such a situation, macrophages can phagocytize normally, but the enveloped organism is not killed. A defect in the plasma membrane enzyme which converts
30 oxygen to lethally reactive forms results in abscess formation in liver, lungs, spleen, lymph nodes, and beneath the skin. Eosinophilia is an excess of eosinophils commonly observed in patients with allergies (hay fever, asthma), allergic reactions to drugs, rheumatoid arthritis, and cancers (Hodgkin's disease, lung, and liver cancer) (Isselbacher, K.J. et al. (1994) Harrison's Principles of Internal Medicine, McGraw-Hill, Inc., New York NY).

Host defense is further augmented by the complement system. The complement system serves as an effector system and is involved in infectious agent recognition. It can function as an independent immune network or in conjunction with other humoral immune responses. The complement system is comprised of numerous plasma and membrane proteins that act in a cascade of reaction sequences whereby one component activates the next. The result is a rapid and amplified response to infection through either an inflammatory response or increased phagocytosis.

The complement system has more than 30 protein components which can be divided into functional groupings including modified serine proteases, membrane-binding proteins and regulators of complement activation. Activation occurs through two different pathways the classical and the alternative. Both pathways serve to destroy infectious agents through distinct triggering mechanisms that eventually merge with the involvement of the component C3.

The classical pathway requires antibody binding to infectious agent antigens. The antibodies serve to define the target and initiate the complement system cascade, culminating in the destruction of the infectious agent. In this pathway, since the antibody guides initiation of the process, the complement can be seen as an effector arm of the humoral immune system.

The alternative pathway of the complement system does not require the presence of pre-existing antibodies for targeting infectious agent destruction. Rather, this pathway, through low levels of an activated component, remains constantly primed and provides surveillance in the non-immune host to enable targeting and destruction of infectious agents. In this case foreign material triggers the cascade, thereby facilitating phagocytosis or lysis (Paul, *supra*, pp.918-919).

Another important component of host defense is the process of inflammation. Inflammatory responses are divided into four categories on the basis of pathology and include allergic inflammation, cytotoxic antibody mediated inflammation, immune complex mediated inflammation and monocyte mediated inflammation. Inflammation manifests as a combination of each of these forms with one predominating.

Allergic acute inflammation is observed in individuals wherein specific antigens stimulate IgE antibody production. Mast cells and basophils are subsequently activated by the attachment of antigen-IgE complexes, resulting in the release of cytoplasmic granule contents such as histamine. The products of activated mast cells can increase vascular permeability and constrict the smooth muscle of breathing passages, resulting in anaphylaxis or asthma. Acute inflammation is also mediated by cytotoxic antibodies and can result in the destruction of tissue through the binding of complement-fixing antibodies to cells. The responsible antibodies are of the IgG or IgM types. Resultant clinical disorders include autoimmune hemolytic anemia and thrombocytopenia as associated with systemic lupus erythematosus.

Immune complex mediated acute inflammation involves the IgG or IgM antibody types which combine with antigen to activate the complement cascade. When such immune complexes bind to neutrophils and macrophages they activate the respiratory burst to form protein- and vessel-damaging agents such as hydrogen peroxide, hydroxyl radical, hypochlorous acid, and chloramines.

5 Clinical manifestations include rheumatoid arthritis and systemic lupus erythematosus.

In chronic inflammation or delayed-type hypersensitivity, macrophages are activated and process antigen for presentation to T cells that subsequently produce lymphokines and monokines. This type of inflammatory response is likely important for defense against intracellular parasites and certain viruses. Clinical associations include, granulomatous disease, tuberculosis, leprosy, and
10 sarcoidosis (Paul, W.E., supra, pp.1017-1018).

Extracellular Information Transmission Molecules

Intercellular communication is essential for the growth and survival of multicellular organisms, and in particular, for the function of the endocrine, nervous, and immune systems. In
15 addition, intercellular communication is critical for developmental processes such as tissue construction and organogenesis, in which cell proliferation, cell differentiation, and morphogenesis must be spatially and temporally regulated in a precise and coordinated manner. Cells communicate with one another through the secretion and uptake of diverse types of signaling molecules such as hormones, growth factors, neuropeptides, and cytokines.

20 Hormones

Hormones are signaling molecules that coordinately regulate basic physiological processes from embryogenesis throughout adulthood. These processes include metabolism, respiration, reproduction, excretion, fetal tissue differentiation and organogenesis, growth and development, homeostasis, and the stress response. Hormonal secretions and the nervous system are tightly
25 integrated and interdependent. Hormones are secreted by endocrine glands, primarily the hypothalamus and pituitary, the thyroid and parathyroid, the pancreas, the adrenal glands, and the ovaries and testes.

The secretion of hormones into the circulation is tightly controlled. Hormones are often secreted in diurnal, pulsatile, and cyclic patterns. Hormone secretion is regulated by perturbations in blood biochemistry, by other upstream-acting hormones, by neural impulses, and by negative feedback
30 loops. Blood hormone concentrations are constantly monitored and adjusted to maintain optimal, steady-state levels. Once secreted, hormones act only on those target cells that express specific receptors.

Most disorders of the endocrine system are caused by either hyposecretion or hypersecretion of hormones. Hyposecretion often occurs when a hormone's gland of origin is damaged or otherwise

impaired. Hypersecretion often results from the proliferation of tumors derived from hormone-secreting cells. Inappropriate hormone levels may also be caused by defects in regulatory feedback loops or in the processing of hormone precursors. Endocrine malfunction may also occur when the target cell fails to respond to the hormone.

5 Hormones can be classified biochemically as polypeptides, steroids, eicosanoids, or amines. Polypeptides, which include diverse hormones such as insulin and growth hormone, vary in size and function and are often synthesized as inactive precursors that are processed intracellularly into mature, active forms. Amines, which include epinephrine and dopamine, are amino acid derivatives that function in neuroendocrine signaling. Steroids, which include the cholesterol-derived hormones
10 estrogen and testosterone, function in sexual development and reproduction. Eicosanoids, which include prostaglandins and prostacyclins, are fatty acid derivatives that function in a variety of processes. Most polypeptides and some amines are soluble in the circulation where they are highly susceptible to proteolytic degradation within seconds after their secretion. Steroids and lipids are insoluble and must be transported in the circulation by carrier proteins. The following discussion will
15 focus primarily on polypeptide hormones.

 Hormones secreted by the hypothalamus and pituitary gland play a critical role in endocrine function by coordinately regulating hormonal secretions from other endocrine glands in response to neural signals. Hypothalamic hormones include thyrotropin-releasing hormone, gonadotropin-releasing hormone, somatostatin, growth-hormone releasing factor, corticotropin-releasing hormone, substance P,
20 dopamine, and prolactin-releasing hormone. These hormones directly regulate the secretion of hormones from the anterior lobe of the pituitary. Hormones secreted by the anterior pituitary include adrenocorticotrophic hormone (ACTH), melanocyte-stimulating hormone, somatotrophic hormones such as growth hormone and prolactin, glycoprotein hormones such as thyroid-stimulating hormone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), β -lipotropin, and β -endorphins.
25 These hormones regulate hormonal secretions from the thyroid, pancreas, and adrenal glands, and act directly on the reproductive organs to stimulate ovulation and spermatogenesis. The posterior pituitary synthesizes and secretes antidiuretic hormone (ADH, vasopressin) and oxytocin.

 Disorders of the hypothalamus and pituitary often result from lesions such as primary brain tumors, adenomas, infarction associated with pregnancy, hypophysectomy, aneurysms, vascular
30 malformations, thrombosis, infections, immunological disorders, and complications due to head trauma. Such disorders have profound effects on the function of other endocrine glands. Disorders associated with hypopituitarism include hypogonadism, Sheehan syndrome, diabetes insipidus, Kallman's disease, Hand-Schuller-Christian disease, Letterer-Siwe disease, sarcoidosis, empty sella syndrome, and dwarfism. Disorders associated with hyperpituitarism include acromegaly, gigantism, and syndrome of

inappropriate ADH secretion (SIADH), often caused by benign adenomas.

Hormones secreted by the thyroid and parathyroid primarily control metabolic rates and the regulation of serum calcium levels, respectively. Thyroid hormones include calcitonin, somatostatin, and thyroid hormone. The parathyroid secretes parathyroid hormone. Disorders associated with
5 hypothyroidism include goiter, myxedema, acute thyroiditis associated with bacterial infection, subacute thyroiditis associated with viral infection, autoimmune thyroiditis (Hashimoto's disease), and cretinism. Disorders associated with hyperthyroidism include thyrotoxicosis and its various forms, Grave's disease, pretibial myxedema, toxic multinodular goiter, thyroid carcinoma, and Plummer's disease. Disorders associated with hyperparathyroidism include Conn disease (chronic hypercalcemia)
10 leading to bone resorption and parathyroid hyperplasia.

Hormones secreted by the pancreas regulate blood glucose levels by modulating the rates of carbohydrate, fat, and protein metabolism. Pancreatic hormones include insulin, glucagon, amylin, γ -aminobutyric acid, gastrin, somatostatin, and pancreatic polypeptide. The principal disorder associated with pancreatic dysfunction is diabetes mellitus caused by insufficient insulin activity. Diabetes
15 mellitus is generally classified as either Type I (insulin-dependent, juvenile diabetes) or Type II (non-insulin-dependent, adult diabetes). The treatment of both forms by insulin replacement therapy is well known. Diabetes mellitus often leads to acute complications such as hypoglycemia (insulin shock), coma, diabetic ketoacidosis, lactic acidosis, and chronic complications leading to disorders of the eye, kidney, skin, bone, joint, cardiovascular system, nervous system, and to decreased resistance to
20 infection.

The anatomy, physiology, and diseases related to hormonal function are reviewed in McCance, K.L. and S.E. Huether (1994) Pathophysiology: The Biological Basis for Disease in Adults and Children, Mosby-Year Book, Inc., St. Louis MO; Greenspan, F.S. and J.D. Baxter (1994) Basic and Clinical Endocrinology, Appleton and Lange, East Norwalk CT.

25 Growth Factors

Growth factors are secreted proteins that mediate intercellular communication. Unlike hormones, which travel great distances via the circulatory system, most growth factors are primarily local mediators that act on neighboring cells. Most growth factors contain a hydrophobic N-terminal signal peptide sequence which directs the growth factor into the secretory pathway. Most growth
30 factors also undergo post-translational modifications within the secretory pathway. These modifications can include proteolysis, glycosylation, phosphorylation, and intramolecular disulfide bond formation. Once secreted, growth factors bind to specific receptors on the surfaces of neighboring target cells, and the bound receptors trigger intracellular signal transduction pathways. These signal transduction pathways elicit specific cellular responses in the target cells. These responses can include

the modulation of gene expression and the stimulation or inhibition of cell division, cell differentiation, and cell motility.

Growth factors fall into at least two broad and overlapping classes. The broadest class includes the large polypeptide growth factors, which are wide-ranging in their effects. These factors include epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor- β (TGF- β), insulin-like growth factor (IGF), nerve growth factor (NGF), and platelet-derived growth factor (PDGF), each defining a family of numerous related factors. The large polypeptide growth factors, with the exception of NGF, act as mitogens on diverse cell types to stimulate wound healing, bone synthesis and remodeling, extracellular matrix synthesis, and proliferation of epithelial, epidermal, and connective tissues. Members of the TGF- β , EGF, and FGF families also function as inductive signals in the differentiation of embryonic tissue. NGF functions specifically as a neurotrophic factor, promoting neuronal growth and differentiation.

Another class of growth factors includes the hematopoietic growth factors, which are narrow in their target specificity. These factors stimulate the proliferation and differentiation of blood cells such as B-lymphocytes, T-lymphocytes, erythrocytes, platelets, eosinophils, basophils, neutrophils, macrophages, and their stem cell precursors. These factors include the colony-stimulating factors (G-CSF, M-CSF, GM-CSF, and CSF1-3), erythropoietin, and the cytokines. The cytokines are specialized hematopoietic factors secreted by cells of the immune system and are discussed in detail below.

Growth factors play critical roles in neoplastic transformation of cells in vitro and in tumor progression in vivo. Overexpression of the large polypeptide growth factors promotes the proliferation and transformation of cells in culture. Inappropriate expression of these growth factors by tumor cells in vivo may contribute to tumor vascularization and metastasis. Inappropriate activity of hematopoietic growth factors can result in anemias, leukemias, and lymphomas. Moreover, growth factors are both structurally and functionally related to oncoproteins, the potentially cancer-causing products of proto-oncogenes. Certain FGF and PDGF family members are themselves homologous to oncoproteins, whereas receptors for some members of the EGF, NGF, and FGF families are encoded by proto-oncogenes. Growth factors also affect the transcriptional regulation of both proto-oncogenes and oncosuppressor genes (Pimentel, E. (1994) Handbook of Growth Factors, CRC Press, Ann Arbor MI; McKay, I. and I. Leigh, eds. (1993) Growth Factors: A Practical Approach, Oxford University Press, New York NY; Habenicht, A., ed. (1990) Growth Factors, Differentiation Factors, and Cytokines, Springer-Verlag, New York NY).

In addition, some of the large polypeptide growth factors play crucial roles in the induction of the primordial germ layers in the developing embryo. This induction ultimately results in the formation of the embryonic mesoderm, ectoderm, and endoderm which in turn provide the framework for the

entire adult body plan. Disruption of this inductive process would be catastrophic to embryonic development.

Small Peptide Factors - Neuropeptides and Vasomediators

Neuropeptides and vasomediators (NP/VM) comprise a family of small peptide factors, typically of 20 amino acids or less. These factors generally function in neuronal excitation and inhibition of vasoconstriction/vasodilation, muscle contraction, and hormonal secretions from the brain and other endocrine tissues. Included in this family are neuropeptides and neuropeptide hormones such as bombesin, neuropeptide Y, neurotensin, neuromedin N, melanocortins, opioids, galanin, somatostatin, tachykinins, urotensin II and related peptides involved in smooth muscle stimulation, vasopressin, vasoactive intestinal peptide, and circulatory system-borne signaling molecules such as angiotensin, complement, calcitonin, endothelins, formyl-methionyl peptides, glucagon, cholecystokinin, gastrin, and many of the peptide hormones discussed above. NP/VMs can transduce signals directly, modulate the activity or release of other neurotransmitters and hormones, and act as catalytic enzymes in signaling cascades. The effects of NP/VMs range from extremely brief to long-lasting. (Reviewed in Martin, C.R. et al. (1985) Endocrine Physiology, Oxford University Press, New York NY, pp. 57-62.)

Cytokines

Cytokines comprise a family of signaling molecules that modulate the immune system and the inflammatory response. Cytokines are usually secreted by leukocytes, or white blood cells, in response to injury or infection. Cytokines function as growth and differentiation factors that act primarily on cells of the immune system such as B- and T-lymphocytes, monocytes, macrophages, and granulocytes. Like other signaling molecules, cytokines bind to specific plasma membrane receptors and trigger intracellular signal transduction pathways which alter gene expression patterns. There is considerable potential for the use of cytokines in the treatment of inflammation and immune system disorders.

Cytokine structure and function have been extensively characterized in vitro. Most cytokines are small polypeptides of about 30 kilodaltons or less. Over 50 cytokines have been identified from human and rodent sources. Examples of cytokine subfamilies include the interferons (IFN- α , - β , and - γ), the interleukins (IL1-IL13), the tumor necrosis factors (TNF- α and - β), and the chemokines. Many cytokines have been produced using recombinant DNA techniques, and the activities of individual cytokines have been determined in vitro. These activities include regulation of leukocyte proliferation, differentiation, and motility.

The activity of an individual cytokine in vitro may not reflect the full scope of that cytokine's activity in vivo. Cytokines are not expressed individually in vivo but are instead expressed in combination with a multitude of other cytokines when the organism is challenged with a stimulus.

Together, these cytokines collectively modulate the immune response in a manner appropriate for that particular stimulus. Therefore, the physiological activity of a cytokine is determined by the stimulus itself and by complex interactive networks among co-expressed cytokines which may demonstrate both synergistic and antagonistic relationships.

5 Chemokines comprise a cytokine subfamily with over 30 members. (Reviewed in Wells, T. N.C. and M.C. Peitsch (1997) *J. Leukoc. Biol.* 61:545-550.) Chemokines were initially identified as chemotactic proteins that recruit monocytes and macrophages to sites of inflammation. Recent evidence indicates that chemokines may also play key roles in hematopoiesis and HIV-1 infection. Chemokines are small proteins which range from about 6-15 kilodaltons in molecular weight. Chemokines are
10 further classified as C, CC, CXC, or CX₃C based on the number and position of critical cysteine residues. The CC chemokines, for example, each contain a conserved motif consisting of two consecutive cysteines followed by two additional cysteines which occur downstream at 24- and 16-residue intervals, respectively (ExPASy PROSITE database, documents PS00472 and PDOC00434). The presence and spacing of these four cysteine residues are highly conserved, whereas the intervening
15 residues diverge significantly. However, a conserved tyrosine located about 15 residues downstream of the cysteine doublet seems to be important for chemotactic activity. Most of the human genes encoding CC chemokines are clustered on chromosome 17, although there are a few examples of CC chemokine genes that map elsewhere. Other chemokines include lymphotactin (C chemokine); macrophage chemotactic and activating factor (MCAF/MCP-1; CC chemokine); platelet factor 4 and IL-8 (CXC
20 chemokines); and fractalkine and neurotractin (CX₃C chemokines). (Reviewed in Luster, A.D. (1998) *N. Engl. J. Med.* 338:436-445.)

Receptor Molecules

The term receptor describes proteins that specifically recognize other molecules. The category
25 is broad and includes proteins with a variety of functions. The bulk of receptors are cell surface proteins which bind extracellular ligands and produce cellular responses in the areas of growth, differentiation, endocytosis, and immune response. Other receptors facilitate the selective transport of proteins out of the endoplasmic reticulum and localize enzymes to particular locations in the cell. The term may also be applied to proteins which act as receptors for ligands with known or unknown
30 chemical composition and which interact with other cellular components. For example, the steroid hormone receptors bind to and regulate transcription of DNA.

Regulation of cell proliferation, differentiation, and migration is important for the formation and function of tissues. Regulatory proteins such as growth factors coordinately control these cellular processes and act as mediators in cell-cell signaling pathways. Growth factors are secreted proteins

that bind to specific cell-surface receptors on target cells. The bound receptors trigger intracellular signal transduction pathways which activate various downstream effectors that regulate gene expression, cell division, cell differentiation, cell motility, and other cellular processes.

Cell surface receptors are typically integral plasma membrane proteins. These receptors
5 recognize hormones such as catecholamines; peptide hormones; growth and differentiation factors;
small peptide factors such as thyrotropin-releasing hormone; galanin, somatostatin, and tachykinins;
and circulatory system-borne signaling molecules. Cell surface receptors on immune system cells
recognize antigens, antibodies, and major histocompatibility complex (MHC)-bound peptides. Other
cell surface receptors bind ligands to be internalized by the cell. This receptor-mediated endocytosis
10 functions in the uptake of low density lipoproteins (LDL), transferrin, glucose- or mannose-terminal
glycoproteins, galactose-terminal glycoproteins, immunoglobulins, phosphovitellogenins, fibrin,
proteinase-inhibitor complexes, plasminogen activators, and thrombospondin (Lodish, H. et al. (1995)
Molecular Cell Biology, Scientific American Books, New York NY, p. 723; Mikhailenko, I. et al.
(1997) J. Biol. Chem. 272:6784-6791).

15 Receptor Protein Kinases

Many growth factor receptors, including receptors for epidermal growth factor,
platelet-derived growth factor, fibroblast growth factor, as well as the growth modulator α -thrombin,
contain intrinsic protein kinase activities. When growth factor binds to the receptor, it triggers the
autophosphorylation of a serine, threonine, or tyrosine residue on the receptor. These phosphorylated
20 sites are recognition sites for the binding of other cytoplasmic signaling proteins. These proteins
participate in signaling pathways that eventually link the initial receptor activation at the cell surface to
the activation of a specific intracellular target molecule. In the case of tyrosine residue
autophosphorylation, these signaling proteins contain a common domain referred to as a Src homology
(SH) domain. SH2 domains and SH3 domains are found in phospholipase C- γ , PI-3-K p85 regulatory
25 subunit, Ras-GTPase activating protein, and pp60^{c-src} (Lowenstein, E.J. et al. (1992) Cell 70:431-442).
The cytokine family of receptors share a different common binding domain and include transmembrane
receptors for growth hormone (GH), interleukins, erythropoietin, and prolactin.

Other receptors and second messenger-binding proteins have intrinsic serine/threonine protein
kinase activity. These include activin/TGF- β /BMP-superfamily receptors, calcium- and diacylglycerol-
30 activated/phospholipid-dependant protein kinase (PK-C), and RNA-dependant protein kinase (PK-R).
In addition, other serine/threonine protein kinases, including nematode Twitchin, have fibronectin-like,
immunoglobulin C2-like domains.

G-Protein Coupled Receptors

G-protein coupled receptors (GPCRs) are integral membrane proteins characterized by the

presence of seven hydrophobic transmembrane domains which span the plasma membrane and form a bundle of antiparallel alpha (α) helices. These proteins range in size from under 400 to over 1000 amino acids (Strosberg, A.D. (1991) *Eur. J. Biochem.* 196:1-10; Coughlin, S.R. (1994) *Curr. Opin. Cell Biol.* 6:191-197). The amino-terminus of the GPCR is extracellular, of variable length and often
5 glycosylated; the carboxy-terminus is cytoplasmic and generally phosphorylated. Extracellular loops of the GPCR alternate with intracellular loops and link the transmembrane domains. The most conserved domains of GPCRs are the transmembrane domains and the first two cytoplasmic loops. The transmembrane domains account for structural and functional features of the receptor. In most cases, the bundle of α helices forms a binding pocket. In addition, the extracellular N-terminal segment or one
10 or more of the three extracellular loops may also participate in ligand binding. Ligand binding activates the receptor by inducing a conformational change in intracellular portions of the receptor. The activated receptor, in turn, interacts with an intracellular heterotrimeric guanine nucleotide binding (G) protein complex which mediates further intracellular signaling activities, generally the production of second messengers such as cyclic AMP (cAMP), phospholipase C, inositol triphosphate, or interactions
15 with ion channel proteins (Baldwin, J.M. (1994) *Curr. Opin. Cell Biol.* 6:180-190).

GPCRs include those for acetylcholine, adenosine, epinephrine and norepinephrine, bombesin, bradykinin, chemokines, dopamine, endothelin, γ -aminobutyric acid (GABA), follicle-stimulating hormone (FSH), glutamate, gonadotropin-releasing hormone (GnRH), hepatocyte growth factor, histamine, leukotrienes, melanocortins, neuropeptide Y, opioid peptides, opsins, prostanoids, serotonin,
20 somatostatin, tachykinins, thrombin, thyrotropin-releasing hormone (TRH), vasoactive intestinal polypeptide family, vasopressin and oxytocin, and orphan receptors.

GPCR mutations, which may cause loss of function or constitutive activation, have been associated with numerous human diseases (Coughlin, *supra*). For instance, retinitis pigmentosa may arise from mutations in the rhodopsin gene. Rhodopsin is the retinal photoreceptor which is located
25 within the discs of the eye rod cell. Parma, J. et al. (1993, *Nature* 365:649-651) report that somatic activating mutations in the thyrotropin receptor cause hyperfunctioning thyroid adenomas and suggest that certain GPCRs susceptible to constitutive activation may behave as protooncogenes.

Nuclear Receptors

Nuclear receptors bind small molecules such as hormones or second messengers, leading to
30 increased receptor-binding affinity to specific chromosomal DNA elements. In addition the affinity for other nuclear proteins may also be altered. Such binding and protein-protein interactions may regulate and modulate gene expression. Examples of such receptors include the steroid hormone receptors family, the retinoic acid receptors family, and the thyroid hormone receptors family.

Ligand-Gated Receptor Ion Channels

Ligand-gated receptor ion channels fall into two categories. The first category, extracellular ligand-gated receptor ion channels (ELGs), rapidly transduce neurotransmitter-binding events into electrical signals, such as fast synaptic neurotransmission. ELG function is regulated by post-translational modification. The second category, intracellular ligand-gated receptor ion channels
5 (ILGs), are activated by many intracellular second messengers and do not require post-translational modification(s) to effect a channel-opening response.

ELGs depolarize excitable cells to the threshold of action potential generation. In non-excitabile cells, ELGs permit a limited calcium ion-influx during the presence of agonist. ELGs include channels directly gated by neurotransmitters such as acetylcholine, L-glutamate, glycine, ATP, serotonin,
10 GABA, and histamine. ELG genes encode proteins having strong structural and functional similarities. ILGs are encoded by distinct and unrelated gene families and include receptors for cAMP, cGMP, calcium ions, ATP, and metabolites of arachidonic acid.

Macrophage Scavenger Receptors

Macrophage scavenger receptors with broad ligand specificity may participate in the binding of
15 low density lipoproteins (LDL) and foreign antigens. Scavenger receptors types I and II are trimeric membrane proteins with each subunit containing a small N-terminal intracellular domain, a transmembrane domain, a large extracellular domain, and a C-terminal cysteine-rich domain. The extracellular domain contains a short spacer domain, an α -helical coiled-coil domain, and a triple helical collagenous domain. These receptors have been shown to bind a spectrum of ligands, including
20 chemically modified lipoproteins and albumin, polyribonucleotides, polysaccharides, phospholipids, and asbestos (Matsumoto, A. et al. (1990) Proc. Natl. Acad. Sci. USA 87:9133-9137; Elomaa, O. et al. (1995) Cell 80:603-609). The scavenger receptors are thought to play a key role in atherogenesis by mediating uptake of modified LDL in arterial walls, and in host defense by binding bacterial endotoxins, bacteria, and protozoa.

T-Cell Receptors

T cells play a dual role in the immune system as effectors and regulators, coupling antigen recognition with the transmission of signals that induce cell death in infected cells and stimulate proliferation of other immune cells. Although a population of T cells can recognize a wide range of different antigens, an individual T cell can only recognize a single antigen and only when it is presented
30 to the T cell receptor (TCR) as a peptide complexed with a major histocompatibility molecule (MHC) on the surface of an antigen presenting cell. The TCR on most T cells consists of immunoglobulin-like integral membrane glycoproteins containing two polypeptide subunits, α and β , of similar molecular weight. Both TCR subunits have an extracellular domain containing both variable and constant regions, a transmembrane domain that traverses the membrane once, and a short intracellular domain

(Saito, H. et al. (1984) *Nature* 309:757-762). The genes for the TCR subunits are constructed through somatic rearrangement of different gene segments. Interaction of antigen in the proper MHC context with the TCR initiates signaling cascades that induce the proliferation, maturation, and function of cellular components of the immune system (Weiss, A. (1991) *Annu. Rev. Genet.* 25:487-510).

- 5 Rearrangements in TCR genes and alterations in TCR expression have been noted in lymphomas, leukemias, autoimmune disorders, and immunodeficiency disorders (Aisenberg, A.C. et al. (1985) *N. Engl. J. Med.* 313:529-533; Weiss, supra).

Intracellular Signaling Molecules

- 10 Intracellular signaling is the general process by which cells respond to extracellular signals (hormones, neurotransmitters, growth and differentiation factors, etc.) through a cascade of biochemical reactions that begins with the binding of a signaling molecule to a cell membrane receptor and ends with the activation of an intracellular target molecule. Intermediate steps in the process involve the activation of various cytoplasmic proteins by phosphorylation via protein kinases,
15 and their deactivation by protein phosphatases, and the eventual translocation of some of these activated proteins to the cell nucleus where the transcription of specific genes is triggered. The intracellular signaling process regulates all types of cell functions including cell proliferation, cell differentiation, and gene transcription, and involves a diversity of molecules including protein kinases and phosphatases, and second messenger molecules, such as cyclic nucleotides, calcium-calmodulin,
20 inositol, and various mitogens, that regulate protein phosphorylation.

Protein Phosphorylation

- Protein kinases and phosphatases play a key role in the intracellular signaling process by controlling the phosphorylation and activation of various signaling proteins. The high energy phosphate for this reaction is generally transferred from the adenosine triphosphate molecule (ATP) to
25 a particular protein by a protein kinase and removed from that protein by a protein phosphatase. Protein kinases are roughly divided into two groups: those that phosphorylate tyrosine residues (protein tyrosine kinases, PTK) and those that phosphorylate serine or threonine residues (serine/threonine kinases, STK). A few protein kinases have dual specificity for serine/threonine and tyrosine residues. Almost all kinases contain a conserved 250-300 amino acid catalytic domain
30 containing specific residues and sequence motifs characteristic of the kinase family (Hardie, G. and S. Hanks (1995) The Protein Kinase Facts Books, Vol I:7-20, Academic Press, San Diego CA).

STKs include the second messenger dependent protein kinases such as the cyclic-AMP dependent protein kinases (PKA), involved in mediating hormone-induced cellular responses; calcium-calmodulin (CaM) dependent protein kinases, involved in regulation of smooth muscle

contraction, glycogen breakdown, and neurotransmission; and the mitogen-activated protein kinases (MAP) which mediate signal transduction from the cell surface to the nucleus via phosphorylation cascades. Altered PKA expression is implicated in a variety of disorders and diseases including cancer, thyroid disorders, diabetes, atherosclerosis, and cardiovascular disease (Isselbacher, K.J. et al. (1994) Harrison's Principles of Internal Medicine, McGraw-Hill, New York NY, pp. 416-431, 1887).

PTKs are divided into transmembrane, receptor PTKs and nontransmembrane, non-receptor PTKs. Transmembrane PTKs are receptors for most growth factors. Non-receptor PTKs lack transmembrane regions and, instead, form complexes with the intracellular regions of cell surface receptors. Receptors that function through non-receptor PTKs include those for cytokines and hormones (growth hormone and prolactin) and antigen-specific receptors on T and B lymphocytes. Many of these PTKs were first identified as the products of mutant oncogenes in cancer cells in which their activation was no longer subject to normal cellular controls. In fact, about one third of the known oncogenes encode PTKs, and it is well known that cellular transformation (oncogenesis) is often accompanied by increased tyrosine phosphorylation activity (Charbonneau, H. and N.K. Tonks (1992) *Annu. Rev. Cell Biol.* 8:463-493).

An additional family of protein kinases previously thought to exist only in procaryotes is the histidine protein kinase family (HPK). HPKs bear little homology with mammalian STKs or PTKs but have distinctive sequence motifs of their own (Davie, J.R. et al. (1995) *J. Biol. Chem.* 270:19861-19867). A histidine residue in the N-terminal half of the molecule (region I) is an autophosphorylation site. Three additional motifs located in the C-terminal half of the molecule include an invariant asparagine residue in region II and two glycine-rich loops characteristic of nucleotide binding domains in regions III and IV. Recently a branched chain alpha-ketoacid dehydrogenase kinase has been found with characteristics of HPK in rat (Davie, supra).

Protein phosphatases regulate the effects of protein kinases by removing phosphate groups from molecules previously activated by kinases. The two principal categories of protein phosphatases are the protein (serine/threonine) phosphatases (PPs) and the protein tyrosine phosphatases (PTPs). PPs dephosphorylate phosphoserine/threonine residues and are important regulators of many cAMP-mediated hormone responses (Cohen, P. (1989) *Annu. Rev. Biochem.* 58:453-508). PTPs reverse the effects of protein tyrosine kinases and play a significant role in cell cycle and cell signaling processes (Charbonneau, supra). As previously noted, many PTKs are encoded by oncogenes, and oncogenesis is often accompanied by increased tyrosine phosphorylation activity. It is therefore possible that PTPs may prevent or reverse cell transformation and the growth of various cancers by controlling the levels of tyrosine phosphorylation in cells. This hypothesis is supported by studies showing that overexpression of PTPs can suppress transformation in cells, and that specific

inhibition of PTPs can enhance cell transformation (Charbonneau, *supra*).

Phospholipid and Inositol-Phosphate Signaling

Inositol phospholipids (phosphoinositides) are involved in an intracellular signaling pathway that begins with binding of a signaling molecule to a G-protein linked receptor in the plasma membrane. This leads to the phosphorylation of phosphatidylinositol (PI) residues on the inner side of the plasma membrane to the biphosphate state (PIP_2) by inositol kinases. Simultaneously, the G-protein linked receptor binding stimulates a trimeric G-protein which in turn activates a phosphoinositide-specific phospholipase C- β . Phospholipase C- β then cleaves PIP_2 into two products, inositol triphosphate (IP_3) and diacylglycerol. These two products act as mediators for separate signaling events. IP_3 diffuses through the plasma membrane to induce calcium release from the endoplasmic reticulum (ER), while diacylglycerol remains in the membrane and helps activate protein kinase C, an STK that phosphorylates selected proteins in the target cell. The calcium response initiated by IP_3 is terminated by the dephosphorylation of IP_3 by specific inositol phosphatases. Cellular responses that are mediated by this pathway are glycogen breakdown in the liver in response to vasopressin, smooth muscle contraction in response to acetylcholine, and thrombin-induced platelet aggregation.

Cyclic Nucleotide Signaling

Cyclic nucleotides (cAMP and cGMP) function as intracellular second messengers to transduce a variety of extracellular signals including hormones, light, and neurotransmitters. In particular, cyclic-AMP dependent protein kinases (PKA) are thought to account for all of the effects of cAMP in most mammalian cells, including various hormone-induced cellular responses. Visual excitation and the phototransmission of light signals in the eye is controlled by cyclic-GMP regulated, Ca^{2+} -specific channels. Because of the importance of cellular levels of cyclic nucleotides in mediating these various responses, regulating the synthesis and breakdown of cyclic nucleotides is an important matter. Thus adenylyl cyclase, which synthesizes cAMP from AMP, is activated to increase cAMP levels in muscle by binding of adrenaline to β -adrenergic receptors, while activation of guanylate cyclase and increased cGMP levels in photoreceptors leads to reopening of the Ca^{2+} -specific channels and recovery of the dark state in the eye. In contrast, hydrolysis of cyclic nucleotides by cAMP and cGMP-specific phosphodiesterases (PDEs) produces the opposite of these and other effects mediated by increased cyclic nucleotide levels. PDEs appear to be particularly important in the regulation of cyclic nucleotides, considering the diversity found in this family of proteins. At least seven families of mammalian PDEs (PDE1-7) have been identified based on substrate specificity and affinity, sensitivity to cofactors, and sensitivity to inhibitory drugs (Beavo, J.A. (1995) *Physiological Reviews* 75:725-748). PDE inhibitors have been found to be particularly

useful in treating various clinical disorders. Rolipram, a specific inhibitor of PDE4, has been used in the treatment of depression, and similar inhibitors are undergoing evaluation as anti-inflammatory agents. Theophylline is a nonspecific PDE inhibitor used in the treatment of bronchial asthma and other respiratory diseases (Banner, K.H. and C.P. Page (1995) Eur. Respir. J. 8:996-1000).

5 G-Protein Signaling

Guanine nucleotide binding proteins (G-proteins) are critical mediators of signal transduction between a particular class of extracellular receptors, the G-protein coupled receptors (GPCR), and intracellular second messengers such as cAMP and Ca^{2+} . G-proteins are linked to the cytosolic side of a GPCR such that activation of the GPCR by ligand binding stimulates binding of the G-protein to
10 GTP, inducing an "active" state in the G-protein. In the active state, the G-protein acts as a signal to trigger other events in the cell such as the increase of cAMP levels or the release of Ca^{2+} into the cytosol from the ER, which, in turn, regulate phosphorylation and activation of other intracellular proteins. Recycling of the G-protein to the inactive state involves hydrolysis of the bound GTP to GDP by a GTPase activity in the G-protein. (See Alberts, B. et al. (1994) Molecular Biology of the
15 Cell, Garland Publishing, Inc., New York NY, pp.734-759.) Two structurally distinct classes of G-proteins are recognized: heterotrimeric G-proteins, consisting of three different subunits, and monomeric, low molecular weight (LMW), G-proteins consisting of a single polypeptide chain.

The three polypeptide subunits of heterotrimeric G-proteins are the α , β , and γ subunits. The α subunit binds and hydrolyzes GTP. The β and γ subunits form a tight complex that anchors the
20 protein to the inner side of the plasma membrane. The β subunits, also known as G- β proteins or β transducins, contain seven tandem repeats of the WD-repeat sequence motif, a motif found in many proteins with regulatory functions. Mutations and variant expression of β transducin proteins are linked with various disorders (Neer, E.J. et al. (1994) Nature 371:297-300; Margottin, F. et al. (1998) Mol. Cell 1:565-574).

25 LMW GTP-proteins are GTPases which regulate cell growth, cell cycle control, protein secretion, and intracellular vesicle interaction. They consist of single polypeptides which, like the α subunit of the heterotrimeric G-proteins, are able to bind and hydrolyze GTP, thus cycling between an inactive and an active state. At least sixty members of the LMW G-protein superfamily have been identified and are currently grouped into the six subfamilies of ras, rho, arf, sar1, ran, and rab.
30 Activated ras genes were initially found in human cancers, and subsequent studies confirmed that ras function is critical in determining whether cells continue to grow or become differentiated. Other members of the LMW G-protein superfamily have roles in signal transduction that vary with the function of the activated genes and the locations of the G-proteins.

Guanine nucleotide exchange factors regulate the activities of LMW G-proteins by

determining whether GTP or GDP is bound. GTPase-activating protein (GAP) binds to GTP-ras and induces it to hydrolyze GTP to GDP. In contrast, guanine nucleotide releasing protein (GNRP) binds to GDP-ras and induces the release of GDP and the binding of GTP.

Other regulators of G-protein signaling (RGS) also exist that act primarily by negatively regulating the G-protein pathway by an unknown mechanism (Druey, K.M. et al. (1996) *Nature* 379:742-746). Some 15 members of the RGS family have been identified. RGS family members are related structurally through similarities in an approximately 120 amino acid region termed the RGS domain and functionally by their ability to inhibit the interleukin (cytokine) induction of MAP kinase in cultured mammalian 293T cells (Druey, *supra*).

10 Calcium Signaling Molecules

Ca^{+2} is another second messenger molecule that is even more widely used as an intracellular mediator than cAMP. Two pathways exist by which Ca^{+2} can enter the cytosol in response to extracellular signals: One pathway acts primarily in nerve signal transduction where Ca^{+2} enters a nerve terminal through a voltage-gated Ca^{+2} channel. The second is a more ubiquitous pathway in which Ca^{+2} is released from the ER into the cytosol in response to binding of an extracellular signaling molecule to a receptor. Ca^{2+} directly activates regulatory enzymes, such as protein kinase C, which trigger signal transduction pathways. Ca^{2+} also binds to specific Ca^{2+} -binding proteins (CBPs) such as calmodulin (CaM) which then activate multiple target proteins in the cell including enzymes, membrane transport pumps, and ion channels. CaM interactions are involved in a multitude of cellular processes including, but not limited to, gene regulation, DNA synthesis, cell cycle progression, mitosis, cytokinesis, cytoskeletal organization, muscle contraction, signal transduction, ion homeostasis, exocytosis, and metabolic regulation (Celio, M.R. et al. (1996) Guidebook to Calcium-binding Proteins, Oxford University Press, Oxford, UK, pp. 15-20). Some CBPs can serve as a storage depot for Ca^{2+} in an inactive state. Calsequestrin is one such CBP that is expressed in isoforms specific to cardiac muscle and skeletal muscle. It is suggested that calsequestrin binds Ca^{2+} in a rapidly exchangeable state that is released during Ca^{2+} -signaling conditions (Celio, M.R. et al. (1996) Guidebook to Calcium-binding Proteins, Oxford University Press, New York NY, pp. 222-224).

Cyclins

30 Cell division is the fundamental process by which all living things grow and reproduce. In most organisms, the cell cycle consists of three principle steps; interphase, mitosis, and cytokinesis. Interphase, involves preparations for cell division, replication of the DNA and production of essential proteins. In mitosis, the nuclear material is divided and separates to opposite sides of the cell. Cytokinesis is the final division and fission of the cell cytoplasm to produce the daughter cells.

The entry and exit of a cell from mitosis is regulated by the synthesis and destruction of a family of activating proteins called cyclins. Cyclins act by binding to and activating a group of cyclin-dependent protein kinases (Cdks) which then phosphorylate and activate selected proteins involved in the mitotic process. Several types of cyclins exist. (Ciechanover, A. (1994) Cell 79:13-21.) Two principle types are mitotic cyclin, or cyclin B, which controls entry of the cell into mitosis, and G1 cyclin, which controls events that drive the cell out of mitosis.

Signal Complex Scaffolding Proteins

Certain proteins in intracellular signaling pathways serve to link or cluster other proteins involved in the signaling cascade. A conserved protein domain called the PDZ domain has been identified in various membrane-associated signaling proteins. This domain has been implicated in receptor and ion channel clustering and in the targeting of multiprotein signaling complexes to specialized functional regions of the cytosolic face of the plasma membrane. (For a review of PDZ domain-containing proteins, see Ponting, C.P. et al. (1997) Bioessays 19:469-479.) A large proportion of PDZ domains are found in the eukaryotic MAGUK (membrane-associated guanylate kinase) protein family, members of which bind to the intracellular domains of receptors and channels. However, PDZ domains are also found in diverse membrane-localized proteins such as protein tyrosine phosphatases, serine/threonine kinases, G-protein cofactors, and synapse-associated proteins such as syntrophins and neuronal nitric oxide synthase (nNOS). Generally, about one to three PDZ domains are found in a given protein, although up to nine PDZ domains have been identified in a single protein.

Membrane Transport Molecules

The plasma membrane acts as a barrier to most molecules. Transport between the cytoplasm and the extracellular environment, and between the cytoplasm and lumenal spaces of cellular organelles requires specific transport proteins. Each transport protein carries a particular class of molecule, such as ions, sugars, or amino acids, and often is specific to a certain molecular species of the class. A variety of human inherited diseases are caused by a mutation in a transport protein. For example, cystinuria is an inherited disease that results from the inability to transport cystine, the disulfide-linked dimer of cysteine, from the urine into the blood. Accumulation of cystine in the urine leads to the formation of cystine stones in the kidneys.

Transport proteins are multi-pass transmembrane proteins, which either actively transport molecules across the membrane or passively allow them to cross. Active transport involves directional pumping of a solute across the membrane, usually against an electrochemical gradient. Active transport is tightly coupled to a source of metabolic energy, such as ATP hydrolysis or an

electrochemically favorable ion gradient. Passive transport involves the movement of a solute down its electrochemical gradient. Transport proteins can be further classified as either carrier proteins or channel proteins. Carrier proteins, which can function in active or passive transport, bind to a specific solute to be transported and undergo a conformational change which transfers the bound solute across the membrane. Channel proteins, which only function in passive transport, form hydrophilic pores across the membrane. When the pores open, specific solutes, such as inorganic ions, pass through the membrane and down the electrochemical gradient of the solute.

Carrier proteins which transport a single solute from one side of the membrane to the other are called uniporters. In contrast, coupled transporters link the transfer of one solute with simultaneous or sequential transfer of a second solute, either in the same direction (symport) or in the opposite direction (antiport). For example, intestinal and kidney epithelium contains a variety of symporter systems driven by the sodium gradient that exists across the plasma membrane. Sodium moves into the cell down its electrochemical gradient and brings the solute into the cell with it. The sodium gradient that provides the driving force for solute uptake is maintained by the ubiquitous Na^+/K^+ ATPase. Sodium-coupled transporters include the mammalian glucose transporter (SGLT1), iodide transporter (NIS), and multivitamin transporter (SMVT). All three transporters have twelve putative transmembrane segments, extracellular glycosylation sites, and cytoplasmically-oriented N- and C-termini. NIS plays a crucial role in the evaluation, diagnosis, and treatment of various thyroid pathologies because it is the molecular basis for radioiodide thyroid-imaging techniques and for specific targeting of radioisotopes to the thyroid gland (Levy, O. et al. (1997) Proc. Natl. Acad. Sci. USA 94:5568-5573). SMVT is expressed in the intestinal mucosa, kidney, and placenta, and is implicated in the transport of the water-soluble vitamins, e.g., biotin and pantothenate (Prasad, P.D. et al. (1998) J. Biol. Chem. 273:7501-7506).

Transporters play a major role in the regulation of pH, excretion of drugs, and the cellular K^+/Na^+ balance. Monocarboxylate anion transporters are proton-coupled symporters with a broad substrate specificity that includes L-lactate, pyruvate, and the ketone bodies acetate, acetoacetate, and beta-hydroxybutyrate. At least seven isoforms have been identified to date. The isoforms are predicted to have twelve transmembrane (TM) helical domains with a large intracellular loop between TM6 and TM7, and play a critical role in maintaining intracellular pH by removing the protons that are produced stoichiometrically with lactate during glycolysis. The best characterized $\text{H}^{(+)}$ -monocarboxylate transporter is that of the erythrocyte membrane, which transports L-lactate and a wide range of other aliphatic monocarboxylates. Other cells possess $\text{H}^{(+)}$ -linked monocarboxylate transporters with differing substrate and inhibitor selectivities. In particular, cardiac muscle and tumor cells have transporters that differ in their K_m values for certain substrates, including stereoselectivity for L- over

D-lactate, and in their sensitivity to inhibitors. There are Na(+)-monocarboxylate cotransporters on the luminal surface of intestinal and kidney epithelia, which allow the uptake of lactate, pyruvate, and ketone bodies in these tissues. In addition, there are specific and selective transporters for organic cations and organic anions in organs including the kidney, intestine and liver. Organic anion

5 transporters are selective for hydrophobic, charged molecules with electron-attracting side groups. Organic cation transporters, such as the ammonium transporter, mediate the secretion of a variety of drugs and endogenous metabolites, and contribute to the maintenance of intercellular pH. (Poole, R.C. and A.P. Halestrap (1993) *Am. J. Physiol.* 264:C761-C782; Price, N.T. et al. (1998) *Biochem. J.* 329:321-328; and Martinelle, K. and I. Haggstrom (1993) *J. Biotechnol.* 30: 339-350.)

10 The largest and most diverse family of transport proteins known is the ATP-binding cassette (ABC) transporters. As a family, ABC transporters can transport substances that differ markedly in chemical structure and size, ranging from small molecules such as ions, sugars, amino acids, peptides, and phospholipids, to lipopeptides, large proteins, and complex hydrophobic drugs. ABC proteins consist of four modules: two nucleotide-binding domains (NBD), which hydrolyze ATP to supply the
15 energy required for transport, and two membrane-spanning domains (MSD), each containing six putative transmembrane segments. These four modules may be encoded by a single gene, as is the case for the cystic fibrosis transmembrane regulator (CFTR), or by separate genes. When encoded by separate genes, each gene product contains a single NBD and MSD. These "half-molecules" form homo- and heterodimers, such as Tap1 and Tap2, the endoplasmic reticulum-based major
20 histocompatibility (MHC) peptide transport system. Several genetic diseases are attributed to defects in ABC transporters, such as the following diseases and their corresponding proteins: cystic fibrosis (CFTR, an ion channel), adrenoleukodystrophy (adrenoleukodystrophy protein, ALDP), Zellweger syndrome (peroxisomal membrane protein-70, PMP70), and hyperinsulinemic hypoglycemia (sulfonylurea receptor, SUR). Overexpression of the multidrug resistance (MDR) protein, another
25 ABC transporter, in human cancer cells makes the cells resistant to a variety of cytotoxic drugs used in chemotherapy (Taglicht, D. and S. Michaelis (1998) *Meth. Enzymol.* 292:131-163).

Transport of fatty acids across the plasma membrane can occur by diffusion, a high capacity, low affinity process. However, under normal physiological conditions a significant fraction of fatty acid transport appears to occur via a high affinity, low capacity protein-mediated transport process.
30 Fatty acid transport protein (FATP), an integral membrane protein with four transmembrane segments, is expressed in tissues exhibiting high levels of plasma membrane fatty acid flux, such as muscle, heart, and adipose. Expression of FATP is upregulated in 3T3-L1 cells during adipose conversion, and expression in COS7 fibroblasts elevates uptake of long-chain fatty acids (Hui, T.Y. et al. (1998) *J. Biol. Chem.* 273:27420-27429).

Ion Channels

The electrical potential of a cell is generated and maintained by controlling the movement of ions across the plasma membrane. The movement of ions requires ion channels, which form an ion-selective pore within the membrane. There are two basic types of ion channels, ion transporters and
5 gated ion channels. Ion transporters utilize the energy obtained from ATP hydrolysis to actively transport an ion against the ion's concentration gradient. Gated ion channels allow passive flow of an ion down the ion's electrochemical gradient under restricted conditions. Together, these types of ion channels generate, maintain, and utilize an electrochemical gradient that is used in 1) electrical impulse conduction down the axon of a nerve cell, 2) transport of molecules into cells against concentration
10 gradients, 3) initiation of muscle contraction, and 4) endocrine cell secretion.

Ion transporters generate and maintain the resting electrical potential of a cell. Utilizing the energy derived from ATP hydrolysis, they transport ions against the ion's concentration gradient. These transmembrane ATPases are divided into three families. The phosphorylated (P) class ion transporters, including $\text{Na}^+\text{-K}^+$ ATPase, $\text{Ca}^{2+}\text{-ATPase}$, and $\text{H}^+\text{-ATPase}$, are activated by a
15 phosphorylation event. P-class ion transporters are responsible for maintaining resting potential distributions such that cytosolic concentrations of Na^+ and Ca^{2+} are low and cytosolic concentration of K^+ is high. The vacuolar (V) class of ion transporters includes H^+ pumps on intracellular organelles, such as lysosomes and Golgi. V-class ion transporters are responsible for generating the low pH within the lumen of these organelles that is required for function. The coupling factor (F) class consists of H^+
20 pumps in the mitochondria. F-class ion transporters utilize a proton gradient to generate ATP from ADP and inorganic phosphate (P_i).

The resting potential of the cell is utilized in many processes involving carrier proteins and gated ion channels. Carrier proteins utilize the resting potential to transport molecules into and out of the cell. Amino acid and glucose transport into many cells is linked to sodium ion co-transport
25 (symport) so that the movement of Na^+ down an electrochemical gradient drives transport of the other molecule up a concentration gradient. Similarly, cardiac muscle links transfer of Ca^{2+} out of the cell with transport of Na^+ into the cell (antiport).

Ion channels share common structural and mechanistic themes. The channel consists of four or five subunits or protein monomers that are arranged like a barrel in the plasma membrane. Each
30 subunit typically consists of six potential transmembrane segments (S1, S2, S3, S4, S5, and S6). The center of the barrel forms a pore lined by α -helices or β -strands. The side chains of the amino acid residues comprising the α -helices or β -strands establish the charge (cation or anion) selectivity of the channel. The degree of selectivity, or what specific ions are allowed to pass through the channel, depends on the diameter of the narrowest part of the pore.

Gated ion channels control ion flow by regulating the opening and closing of pores. These channels are categorized according to the manner of regulating the gating function. Mechanically-gated channels open pores in response to mechanical stress, voltage-gated channels open pores in response to changes in membrane potential, and ligand-gated channels open pores in the presence of a specific ion, nucleotide, or neurotransmitter.

Voltage-gated Na^+ and K^+ channels are necessary for the function of electrically excitable cells, such as nerve and muscle cells. Action potentials, which lead to neurotransmitter release and muscle contraction, arise from large, transient changes in the permeability of the membrane to Na^+ and K^+ ions. Depolarization of the membrane beyond the threshold level opens voltage-gated Na^+ channels. Sodium ions flow into the cell, further depolarizing the membrane and opening more voltage-gated Na^+ channels, which propagates the depolarization down the length of the cell. Depolarization also opens voltage-gated potassium channels. Consequently, potassium ions flow outward, which leads to repolarization of the membrane. Voltage-gated channels utilize charged residues in the fourth transmembrane segment (S4) to sense voltage change. The open state lasts only about 1 millisecond, at which time the channel spontaneously converts into an inactive state that cannot be opened irrespective of the membrane potential. Inactivation is mediated by the channel's N-terminus, which acts as a plug that closes the pore. The transition from an inactive to a closed state requires a return to resting potential.

Voltage-gated Na^+ channels are heterotrimeric complexes composed of a 260 kDa pore forming α subunit that associates with two smaller auxiliary subunits, $\beta 1$ and $\beta 2$. The $\beta 2$ subunit is an integral membrane glycoprotein that contains an extracellular Ig domain, and its association with α and $\beta 1$ subunits correlates with increased functional expression of the channel, a change in its gating properties, and an increase in whole cell capacitance due to an increase in membrane surface area. (Isom, L.L. et al. (1995) Cell 83:433-442.)

Voltage-gated Ca^{2+} channels are involved in presynaptic neurotransmitter release, and heart and skeletal muscle contraction. The voltage-gated Ca^{2+} channels from skeletal muscle (L-type) and brain (N-type) have been purified, and though their functions differ dramatically, they have similar subunit compositions. The channels are composed of three subunits. The α_1 subunit forms the membrane pore and voltage sensor, while the $\alpha_2\delta$ and β subunits modulate the voltage-dependence, gating properties, and the current amplitude of the channel. These subunits are encoded by at least six α_1 , one $\alpha_2\delta$, and four β genes. A fourth subunit, γ , has been identified in skeletal muscle. (Walker, D. et al. (1998) J. Biol. Chem. 273:2361-2367; and Jay, S.D. et al. (1990) Science 248:490-492.)

Chloride channels are necessary in endocrine secretion and in regulation of cytosolic and organelle pH. In secretory epithelial cells, Cl^- enters the cell across a basolateral membrane through an

Na^+ , K^+/Cl^- cotransporter, accumulating in the cell above its electrochemical equilibrium concentration. Secretion of Cl^- from the apical surface, in response to hormonal stimulation, leads to flow of Na^+ and water into the secretory lumen. The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel encoded by the gene for cystic fibrosis, a common fatal genetic disorder in humans.

- 5 Loss of CFTR function decreases transepithelial water secretion and, as a result, the layers of mucus that coat the respiratory tree, pancreatic ducts, and intestine are dehydrated and difficult to clear. The resulting blockage of these sites leads to pancreatic insufficiency, "meconium ileus", and devastating "chronic obstructive pulmonary disease" (Al-Awqati, Q. et al. (1992) J. Exp. Biol. 172:245-266).

- Many intracellular organelles contain H^+ -ATPase pumps that generate transmembrane pH and
10 electrochemical differences by moving protons from the cytosol to the organelle lumen. If the membrane of the organelle is permeable to other ions, then the electrochemical gradient can be abrogated without affecting the pH differential. In fact, removal of the electrochemical barrier allows more H^+ to be pumped across the membrane, increasing the pH differential. Cl^- is the sole counterion of H^+ translocation in a number of organelles, including chromaffin granules, Golgi vesicles,
15 lysosomes, and endosomes. Functions that require a low vacuolar pH include uptake of small molecules such as biogenic amines in chromaffin granules, processing of vacuolar constituents such as pro-hormones by proteolytic enzymes, and protein degradation in lysosomes (Al-Awqati, *supra*).

- Ligand-gated channels open their pores when an extracellular or intracellular mediator binds to the channel. Neurotransmitter-gated channels are channels that open when a neurotransmitter binds to
20 their extracellular domain. These channels exist in the postsynaptic membrane of nerve or muscle cells. There are two types of neurotransmitter-gated channels. Sodium channels open in response to excitatory neurotransmitters, such as acetylcholine, glutamate, and serotonin. This opening causes an influx of Na^+ and produces the initial localized depolarization that activates the voltage-gated channels and starts the action potential. Chloride channels open in response to inhibitory neurotransmitters, such
25 as γ -aminobutyric acid (GABA) and glycine, leading to hyperpolarization of the membrane and the subsequent generation of an action potential.

- Ligand-gated channels can be regulated by intracellular second messengers. Calcium-activated K^+ channels are gated by internal calcium ions. In nerve cells, an influx of calcium during depolarization opens K^+ channels to modulate the magnitude of the action potential (Ishi, T.M. et al.
30 (1997) Proc. Natl. Acad. Sci. USA 94:11651-11656). Cyclic nucleotide-gated (CNG) channels are gated by cytosolic cyclic nucleotides. The best examples of these are the cAMP-gated Na^+ channels involved in olfaction and the cGMP-gated cation channels involved in vision. Both systems involve ligand-mediated activation of a G-protein coupled receptor which then alters the level of cyclic nucleotide within the cell.

Ion channels are expressed in a number of tissues where they are implicated in a variety of processes. CNG channels, while abundantly expressed in photoreceptor and olfactory sensory cells, are also found in kidney, lung, pineal, retinal ganglion cells, testis, aorta, and brain. Calcium-activated K^+ channels may be responsible for the vasodilatory effects of bradykinin in the kidney and for shunting
5 excess K^+ from brain capillary endothelial cells into the blood. They are also implicated in repolarizing granulocytes after agonist-stimulated depolarization (Ishi, *supra*). Ion channels have been the target for many drug therapies. Neurotransmitter-gated channels have been targeted in therapies for treatment of insomnia, anxiety, depression, and schizophrenia. Voltage-gated channels have been targeted in therapies for arrhythmia, ischemic stroke, head trauma, and neurodegenerative disease (Taylor, C.P.
10 and L.S. Narasimhan (1997) *Adv. Pharmacol.* 39:47-98).

Disease Correlation

The etiology of numerous human diseases and disorders can be attributed to defects in the transport of molecules across membranes. Defects in the trafficking of membrane-bound transporters and ion channels are associated with several disorders, e.g. cystic fibrosis, glucose-galactose
15 malabsorption syndrome, hypercholesterolemia, von Gierke disease, and certain forms of diabetes mellitus. Single-gene defect diseases resulting in an inability to transport small molecules across membranes include, e.g., cystinuria, iminoglycinuria, Hartup disease, and Fanconi disease (van't Hoff, W.G. (1996) *Exp. Nephrol.* 4:253-262; Talente, G.M. et al. (1994) *Ann. Intern. Med.* 120:218-226; and Chillon, M. et al. (1995) *New Engl. J. Med.* 332:1475-1480).

20

Protein Modification and Maintenance Molecules

The cellular processes regulating modification and maintenance of protein molecules coordinate their conformation, stabilization, and degradation. Each of these processes is mediated by key enzymes or proteins such as proteases, protease inhibitors, transferases, isomerases, and
25 molecular chaperones.

Proteases

Proteases cleave proteins and peptides at the peptide bond that forms the backbone of the peptide and protein chain. Proteolytic processing is essential to cell growth, differentiation, remodeling, and homeostasis as well as inflammation and immune response. Typical protein half-
30 lives range from hours to a few days, so that within all living cells, precursor proteins are being cleaved to their active form, signal sequences proteolytically removed from targeted proteins, and aged or defective proteins degraded by proteolysis. Proteases function in bacterial, parasitic, and viral invasion and replication within a host. Four principal categories of mammalian proteases have been identified based on active site structure, mechanism of action, and overall three-dimensional structure.

(Beynon, R.J. and J.S. Bond (1994) Proteolytic Enzymes: A Practical Approach, Oxford University Press, New York NY, pp. 1-5).

The serine proteases (SPs) have a serine residue, usually within a conserved sequence, in an active site composed of the serine, an aspartate, and a histidine residue. SPs include the digestive enzymes trypsin and chymotrypsin, components of the complement cascade and the blood-clotting cascade, and enzymes that control extracellular protein degradation. The main SP sub-families are trypases, which cleave after arginine or lysine; aspartases, which cleave after aspartate; chymases, which cleave after phenylalanine or leucine; metases, which cleavage after methionine; and serases which cleave after serine. Enterokinase, the initiator of intestinal digestion, is a serine protease found in the intestinal brush border, where it cleaves the acidic propeptide from trypsinogen to yield active trypsin (Kitamoto, Y. et al. (1994) Proc. Natl. Acad. Sci. USA 91:7588-7592).

Prolylcarboxypeptidase, a lysosomal serine peptidase that cleaves peptides such as angiotensin II and III and [des-Arg9] bradykinin, shares sequence homology with members of both the serine carboxypeptidase and prolylendopeptidase families (Tan, F. et al. (1993) J. Biol. Chem. 268:16631-16638).

Cysteine proteases (CPs) have a cysteine as the major catalytic residue at an active site where catalysis proceeds via an intermediate thiol ester and is facilitated by adjacent histidine and aspartic acid residues. CPs are involved in diverse cellular processes ranging from the processing of precursor proteins to intracellular degradation. Mammalian CPs include lysosomal cathepsins and cytosolic calcium activated proteases, calpains. CPs are produced by monocytes, macrophages and other cells of the immune system which migrate to sites of inflammation and secrete molecules involved in tissue repair. Overabundance of these repair molecules plays a role in certain disorders. In autoimmune diseases such as rheumatoid arthritis, secretion of the cysteine peptidase cathepsin C degrades collagen, laminin, elastin and other structural proteins found in the extracellular matrix of bones.

Aspartic proteases are members of the cathepsin family of lysosomal proteases and include pepsin A, gastricsin, chymosin, renin, and cathepsins D and E. Aspartic proteases have a pair of aspartic acid residues in the active site, and are most active in the pH 2 - 3 range, in which one of the aspartate residues is ionized, the other un-ionized. Aspartic proteases include bacterial penicillopepsin, mammalian pepsin, renin, chymosin, and certain fungal proteases. Abnormal regulation and expression of cathepsins is evident in various inflammatory disease states. In cells isolated from inflamed synovia, the mRNA for stromelysin, cytokines, TIMP-1, cathepsin, gelatinase, and other molecules is preferentially expressed. Expression of cathepsins L and D is elevated in synovial tissues from patients with rheumatoid arthritis and osteoarthritis. Cathepsin L expression may

also contribute to the influx of mononuclear cells which exacerbates the destruction of the rheumatoid synovium. (Keyszer, G.M. (1995) *Arthritis Rheum.* 38:976-984.) The increased expression and differential regulation of the cathepsins are linked to the metastatic potential of a variety of cancers and as such are of therapeutic and prognostic interest (Chambers, A.F. et al. (1993) *Crit. Rev. Oncog.* 4:95-114).

5 Metalloproteases have active sites that include two glutamic acid residues and one histidine residue that serve as binding sites for zinc. Carboxypeptidases A and B are the principal mammalian metalloproteases. Both are exoproteases of similar structure and active sites. Carboxypeptidase A, like chymotrypsin, prefers C-terminal aromatic and aliphatic side chains of hydrophobic nature, 10 whereas carboxypeptidase B is directed toward basic arginine and lysine residues. Glycoprotease (GCP), or O-sialoglycoprotein endopeptidase, is a metallopeptidase which specifically cleaves O-sialoglycoproteins such as glycophorin A. Another metallopeptidase, placental leucine aminopeptidase (P-LAP) degrades several peptide hormones such as oxytocin and vasopressin, suggesting a role in maintaining homeostasis during pregnancy, and is expressed in several tissues 15 (Rogi, T. et al. (1996) *J. Biol. Chem.* 271:56-61).

Ubiquitin proteases are associated with the ubiquitin conjugation system (UCS), a major pathway for the degradation of cellular proteins in eukaryotic cells and some bacteria. The UCS mediates the elimination of abnormal proteins and regulates the half-lives of important regulatory proteins that control cellular processes such as gene transcription and cell cycle progression. In the 20 UCS pathway, proteins targeted for degradation are conjugated to a ubiquitin, a small heat stable protein. The ubiquitinated protein is then recognized and degraded by proteasome, a large, multisubunit proteolytic enzyme complex, and ubiquitin is released for reutilization by ubiquitin protease. The UCS is implicated in the degradation of mitotic cyclic kinases, oncoproteins, tumor suppressor genes such as p53, viral proteins, cell surface receptors associated with signal 25 transduction, transcriptional regulators, and mutated or damaged proteins (Ciechanover, A. (1994) *Cell* 79:13-21). A murine proto-oncogene, Unp, encodes a nuclear ubiquitin protease whose overexpression leads to oncogenic transformation of NIH3T3 cells, and the human homolog of this gene is consistently elevated in small cell tumors and adenocarcinomas of the lung (Gray, D.A. (1995) *Oncogene* 10:2179-2183).

30 Signal Peptidases

The mechanism for the translocation process into the endoplasmic reticulum (ER) involves the recognition of an N-terminal signal peptide on the elongating protein. The signal peptide directs the protein and attached ribosome to a receptor on the ER membrane. The polypeptide chain passes through a pore in the ER membrane into the lumen while the N-terminal signal peptide remains

attached at the membrane surface. The process is completed when signal peptidase located inside the ER cleaves the signal peptide from the protein and releases the protein into the lumen.

Protease Inhibitors

Protease inhibitors and other regulators of protease activity control the activity and effects of proteases. Protease inhibitors have been shown to control pathogenesis in animal models of proteolytic disorders (Murphy, G. (1991) Agents Actions Suppl. 35:69-76). Low levels of the cystatins, low molecular weight inhibitors of the cysteine proteases, correlate with malignant progression of tumors. (Calkins, C. et al (1995) Biol. Biochem. Hoppe Seyler 376:71-80). Serpins are inhibitors of mammalian plasma serine proteases. Many serpins serve to regulate the blood clotting cascade and/or the complement cascade in mammals. Sp32 is a positive regulator of the mammalian acrosomal protease, acrosin, that binds the proenzyme, proacrosin, and thereby aides in packaging the enzyme into the acrosomal matrix (Baba, T. et al. (1994) J. Biol. Chem. 269:10133-10140). The Kunitz family of serine protease inhibitors are characterized by one or more "Kunitz domains" containing a series of cysteine residues that are regularly spaced over approximately 50 amino acid residues and form three intrachain disulfide bonds. Members of this family include aprotinin, tissue factor pathway inhibitor (TFPI-1 and TFPI-2), inter- α -trypsin inhibitor, and bikunin. (Marlor, C.W. et al. (1997) J. Biol. Chem. 272:12202-12208.) Members of this family are potent inhibitors (in the nanomolar range) against serine proteases such as kallikrein and plasmin. Aprotinin has clinical utility in reduction of perioperative blood loss.

A major portion of all proteins synthesized in eukaryotic cells are synthesized on the cytosolic surface of the endoplasmic reticulum (ER). Before these immature proteins are distributed to other organelles in the cell or are secreted, they must be transported into the interior lumen of the ER where post-translational modifications are performed. These modifications include protein folding and the formation of disulfide bonds, and N-linked glycosylations.

Protein Isomerases

Protein folding in the ER is aided by two principal types of protein isomerases, protein disulfide isomerase (PDI), and peptidyl-prolyl isomerase (PPI). PDI catalyzes the oxidation of free sulfhydryl groups in cysteine residues to form intramolecular disulfide bonds in proteins. PPI, an enzyme that catalyzes the isomerization of certain proline imidic bonds in oligopeptides and proteins, is considered to govern one of the rate limiting steps in the folding of many proteins to their final functional conformation. The cyclophilins represent a major class of PPI that was originally identified as the major receptor for the immunosuppressive drug cyclosporin A (Handschumacher, R.E. et al. (1984) Science 226: 544-547).

Protein Glycosylation

The glycosylation of most soluble secreted and membrane-bound proteins by oligosaccharides linked to asparagine residues in proteins is also performed in the ER. This reaction is catalyzed by a membrane-bound enzyme, oligosaccharyl transferase. Although the exact purpose of this "N-linked" glycosylation is unknown, the presence of oligosaccharides tends to make a glycoprotein resistant to protease digestion. In addition, oligosaccharides attached to cell-surface proteins called selectins are known to function in cell-cell adhesion processes (Alberts, B. et al. (1994) Molecular Biology of the Cell, Garland Publishing Co., New York NY, p.608). "O-linked" glycosylation of proteins also occurs in the ER by the addition of N-acetylgalactosamine to the hydroxyl group of a serine or threonine residue followed by the sequential addition of other sugar residues to the first. This process is catalysed by a series of glycosyltransferases each specific for a particular donor sugar nucleotide and acceptor molecule (Lodish, H. et al. (1995) Molecular Cell Biology, W.H. Freeman and Co., New York NY, pp.700-708). In many cases, both N- and O-linked oligosaccharides appear to be required for the secretion of proteins or the movement of plasma membrane glycoproteins to the cell surface.

An additional glycosylation mechanism operates in the ER specifically to target lysosomal enzymes to lysosomes and prevent their secretion. Lysosomal enzymes in the ER receive an N-linked oligosaccharide, like plasma membrane and secreted proteins, but are then phosphorylated on one or two mannose residues. The phosphorylation of mannose residues occurs in two steps, the first step being the addition of an N-acetylglucosamine phosphate residue by N-acetylglucosamine phosphotransferase, and the second the removal of the N-acetylglucosamine group by phosphodiesterase. The phosphorylated mannose residue then targets the lysosomal enzyme to a mannose 6-phosphate receptor which transports it to a lysosome vesicle (Lodish, supra, pp. 708-711).

Chaperones

Molecular chaperones are proteins that aid in the proper folding of immature proteins and refolding of improperly folded ones, the assembly of protein subunits, and in the transport of unfolded proteins across membranes. Chaperones are also called heat-shock proteins (hsp) because of their tendency to be expressed in dramatically increased amounts following brief exposure of cells to elevated temperatures. This latter property most likely reflects their need in the refolding of proteins that have become denatured by the high temperatures. Chaperones may be divided into several classes according to their location, function, and molecular weight, and include hsp60, TCP1, hsp70, hsp40 (also called DnaJ), and hsp90. For example, hsp90 binds to steroid hormone receptors, represses transcription in the absence of the ligand, and provides proper folding of the ligand-binding domain of the receptor in the presence of the hormone (Burston, S.G. and A.R. Clarke (1995) Essays Biochem. 29:125-136). Hsp60 and hsp70 chaperones aid in the transport and folding of newly

synthesized proteins. Hsp70 acts early in protein folding, binding a newly synthesized protein before it leaves the ribosome and transporting the protein to the mitochondria or ER before releasing the folded protein. Hsp60, along with hsp10, binds misfolded proteins and gives them the opportunity to refold correctly. All chaperones share an affinity for hydrophobic patches on incompletely folded proteins and the ability to hydrolyze ATP. The energy of ATP hydrolysis is used to release the hsp-bound protein in its properly folded state (Alberts, supra, pp 214, 571-572).

Nucleic Acid Synthesis and Modification Molecules

Polymerases

10 DNA and RNA replication are critical processes for cell replication and function. DNA and RNA replication are mediated by the enzymes DNA and RNA polymerase, respectively, by a "templating" process in which the nucleotide sequence of a DNA or RNA strand is copied by complementary base-pairing into a complementary nucleic acid sequence of either DNA or RNA. However, there are fundamental differences between the two processes.

15 DNA polymerase catalyzes the stepwise addition of a deoxyribonucleotide to the 3'-OH end of a polynucleotide strand (the primer strand) that is paired to a second (template) strand. The new DNA strand therefore grows in the 5' to 3' direction (Alberts, B. et al. (1994)The Molecular Biology of the Cell, Garland Publishing Inc., New York NY, pp. 251-254). The substrates for the polymerization reaction are the corresponding deoxynucleotide triphosphates which must base-pair
20 with the correct nucleotide on the template strand in order to be recognized by the polymerase. Because DNA exists as a double-stranded helix, each of the two strands may serve as a template for the formation of a new complementary strand. Each of the two daughter cells of the dividing cell therefore inherits a new DNA double helix containing one old and one new strand. Thus, DNA is said to be replicated "semiconservatively" by DNA polymerase. In addition to the synthesis of new
25 DNA, DNA polymerase is also involved in the repair of damaged DNA as discussed below under "Ligases."

In contrast to DNA polymerase, RNA polymerase uses a DNA template strand to "transcribe" DNA into RNA using ribonucleotide triphosphates as substrates. Like DNA polymerization, RNA polymerization proceeds in a 5' to 3' direction by addition of a ribonucleoside monophosphate to the
30 3'-OH end of a growing RNA chain. DNA transcription generates messenger RNAs (mRNA) that carry information for protein synthesis, as well as the transfer, ribosomal, and other RNAs that have structural or catalytic functions. In eukaryotes, three discrete RNA polymerases synthesize the three different types of RNA (Alberts, supra, pp. 367-368). RNA polymerase I makes the large ribosomal RNAs, RNA polymerase II makes the mRNAs that will be translated into proteins, and RNA

polymerase III makes a variety of small, stable RNAs, including 5S ribosomal RNA and the transfer RNAs (tRNA). In all cases, RNA synthesis is initiated by binding of the RNA polymerase to a promoter region on the DNA and synthesis begins at a start site within the promoter. Synthesis is completed at a broad, general stop or termination region in the DNA where both the polymerase and the completed RNA chain are released.

Ligases

DNA repair is the process by which accidental base changes, such as those produced by oxidative damage, hydrolytic attack, or uncontrolled methylation of DNA are corrected before replication or transcription of the DNA can occur. Because of the efficiency of the DNA repair process, fewer than one in one thousand accidental base changes causes a mutation (Alberts, *supra*, pp. 245-249). The three steps common to most types of DNA repair are (1) excision of the damaged or altered base or nucleotide by DNA nucleases, leaving a gap; (2) insertion of the correct nucleotide in this gap by DNA polymerase using the complementary strand as the template; and (3) sealing the break left between the inserted nucleotide(s) and the existing DNA strand by DNA ligase. In the last reaction, DNA ligase uses the energy from ATP hydrolysis to activate the 5' end of the broken phosphodiester bond before forming the new bond with the 3'-OH of the DNA strand. In Bloom's syndrome, an inherited human disease, individuals are partially deficient in DNA ligation and consequently have an increased incidence of cancer (Alberts, *supra*, p. 247).

Nucleases

Nucleases comprise both enzymes that hydrolyze DNA (DNase) and RNA (RNase). They serve different purposes in nucleic acid metabolism. Nucleases hydrolyze the phosphodiester bonds between adjacent nucleotides either at internal positions (endonucleases) or at the terminal 3' or 5' nucleotide positions (exonucleases). A DNA exonuclease activity in DNA polymerase, for example, serves to remove improperly paired nucleotides attached to the 3'-OH end of the growing DNA strand by the polymerase and thereby serves a "proofreading" function. As mentioned above, DNA endonuclease activity is involved in the excision step of the DNA repair process.

RNases also serve a variety of functions. For example, RNase P is a ribonucleoprotein enzyme which cleaves the 5' end of pre-tRNAs as part of their maturation process. RNase H digests the RNA strand of an RNA/DNA hybrid. Such hybrids occur in cells invaded by retroviruses, and RNase H is an important enzyme in the retroviral replication cycle. Pancreatic RNase secreted by the pancreas into the intestine hydrolyzes RNA present in ingested foods. RNase activity in serum and cell extracts is elevated in a variety of cancers and infectious diseases (Schein, C.H. (1997) *Nat. Biotechnol.* 15:529-536). Regulation of RNase activity is being investigated as a means to control tumor angiogenesis, allergic reactions, viral infection and replication, and fungal infections.

Methylases

Methylation of specific nucleotides occurs in both DNA and RNA, and serves different functions in the two macromolecules. Methylation of cytosine residues to form 5-methyl cytosine in DNA occurs specifically at CG sequences which are base-paired with one another in the DNA double-helix. This pattern of methylation is passed from generation to generation during DNA replication by an enzyme called "maintenance methylase" that acts preferentially on those CG sequences that are base-paired with a CG sequence that is already methylated. Such methylation appears to distinguish active from inactive genes by preventing the binding of regulatory proteins that "turn on" the gene, but permit the binding of proteins that inactivate the gene (Alberts, *supra*, pp. 448-451). In RNA metabolism, "tRNA methylase" produces one of several nucleotide modifications in tRNA that affect the conformation and base-pairing of the molecule and facilitate the recognition of the appropriate mRNA codons by specific tRNAs. The primary methylation pattern is the dimethylation of guanine residues to form N,N-dimethyl guanine.

Helicases and Single-Stranded Binding Proteins

Helicases are enzymes that destabilize and unwind double helix structures in both DNA and RNA. Since DNA replication occurs more or less simultaneously on both strands, the two strands must first separate to generate a replication "fork" for DNA polymerase to act on. Two types of replication proteins contribute to this process, DNA helicases and single-stranded binding proteins. DNA helicases hydrolyze ATP and use the energy of hydrolysis to separate the DNA strands. Single-stranded binding proteins (SSBs) then bind to the exposed DNA strands without covering the bases, thereby temporarily stabilizing them for templating by the DNA polymerase (Alberts, *supra*, pp. 255-256).

RNA helicases also alter and regulate RNA conformation and secondary structure. Like the DNA helicases, RNA helicases utilize energy derived from ATP hydrolysis to destabilize and unwind RNA duplexes. The most well-characterized and ubiquitous family of RNA helicases is the DEAD-box family, so named for the conserved B-type ATP-binding motif which is diagnostic of proteins in this family. Over 40 DEAD-box helicases have been identified in organisms as diverse as bacteria, insects, yeast, amphibians, mammals, and plants. DEAD-box helicases function in diverse processes such as translation initiation, splicing, ribosome assembly, and RNA editing, transport, and stability. Some DEAD-box helicases play tissue- and stage-specific roles in spermatogenesis and embryogenesis. Overexpression of the DEAD-box 1 protein (DDX1) may play a role in the progression of neuroblastoma (Nb) and retinoblastoma (Rb) tumors (Godbout, R. et al. (1998) J. Biol. Chem. 273:21161-21168). These observations suggest that DDX1 may promote or enhance tumor progression by altering the normal secondary structure and expression levels of RNA in cancer cells.

Other DEAD-box helicases have been implicated either directly or indirectly in tumorigenesis (Discussed in Godbout, supra). For example, murine p68 is mutated in ultraviolet light-induced tumors, and human DDX6 is located at a chromosomal breakpoint associated with B-cell lymphoma. Similarly, a chimeric protein comprised of DDX10 and NUP98, a nucleoporin protein, may be
5 involved in the pathogenesis of certain myeloid malignancies.

Topoisomerases

Besides the need to separate DNA strands prior to replication, the two strands must be "unwound" from one another prior to their separation by DNA helicases. This function is performed by proteins known as DNA topoisomerases. DNA topoisomerase effectively acts as a reversible
10 nuclease that hydrolyzes a phosphodiesterase bond in a DNA strand, permitting the two strands to rotate freely about one another to remove the strain of the helix, and then rejoins the original phosphodiester bond between the two strands. Two types of DNA topoisomerase exist, types I and II. DNA Topoisomerase I causes a single-strand break in a DNA helix to allow the rotation of the two strands of the helix about the remaining phosphodiester bond in the opposite strand. DNA
15 topoisomerase II causes a transient break in both strands of a DNA helix where two double helices cross over one another. This type of topoisomerase can efficiently separate two interlocked DNA circles (Alberts, supra, pp.260-262). Type II topoisomerases are largely confined to proliferating cells in eukaryotes, such as cancer cells. For this reason they are targets for anticancer drugs. Topoisomerase II has been implicated in multi-drug resistance (MDR) as it appears to aid in the repair
20 of DNA damage inflicted by DNA binding agents such as doxorubicin and vincristine.

Recombinases

Genetic recombination is the process of rearranging DNA sequences within an organism's genome to provide genetic variation for the organism in response to changes in the environment. DNA recombination allows variation in the particular combination of genes present in an individual's
25 genome, as well as the timing and level of expression of these genes (see Alberts, supra, pp. 263-273). Two broad classes of genetic recombination are commonly recognized, general recombination and site-specific recombination. General recombination involves genetic exchange between any homologous pair of DNA sequences usually located on two copies of the same chromosome. The process is aided by enzymes called recombinases that "nick" one strand of a DNA duplex more or less
30 randomly and permit exchange with the complementary strand of another duplex. The process does not normally change the arrangement of genes on a chromosome. In site-specific recombination, the recombinase recognizes specific nucleotide sequences present in one or both of the recombining molecules. Base-pairing is not involved in this form of recombination and therefore does not require DNA homology between the recombining molecules. Unlike general recombination, this form of

recombination can alter the relative positions of nucleotide sequences in chromosomes.

Splicing Factors

Various proteins are necessary for processing of transcribed RNAs in the nucleus. Pre-mRNA processing steps include capping at the 5' end with methylguanosine, polyadenylating the 3' end, and splicing to remove introns. The primary RNA transcript from DNA is a faithful copy of the gene containing both exon and intron sequences, and the latter sequences must be cut out of the RNA transcript to produce an mRNA that codes for a protein. This "splicing" of the mRNA sequence takes place in the nucleus with the aid of a large, multicomponent ribonucleoprotein complex known as a spliceosome. The spliceosomal complex is composed of five small nuclear ribonucleoprotein particles (snRNPs) designated U1, U2, U4, U5, and U6, and a number of additional proteins. Each snRNP contains a single species of snRNA and about ten proteins. The RNA components of some snRNPs recognize and base pair with intron consensus sequences. The protein components mediate spliceosome assembly and the splicing reaction. Autoantibodies to snRNP proteins are found in the blood of patients with systemic lupus erythematosus (Stryer, L. (1995) Biochemistry, W.H. Freeman and Company, New York NY, p. 863).

Adhesion Molecules

The surface of a cell is rich in transmembrane proteoglycans, glycoproteins, glycolipids, and receptors. These macromolecules mediate adhesion with other cells and with components of the extracellular matrix (ECM). The interaction of the cell with its surroundings profoundly influences cell shape, strength, flexibility, motility, and adhesion. These dynamic properties are intimately associated with signal transduction pathways controlling cell proliferation and differentiation, tissue construction, and embryonic development.

Cadherins

Cadherins comprise a family of calcium-dependent glycoproteins that function in mediating cell-cell adhesion in virtually all solid tissues of multicellular organisms. These proteins share multiple repeats of a cadherin-specific motif, and the repeats form the folding units of the cadherin extracellular domain. Cadherin molecules cooperate to form focal contacts, or adhesion plaques, between adjacent epithelial cells. The cadherin family includes the classical cadherins and protocadherins. Classical cadherins include the E-cadherin, N-cadherin, and P-cadherin subfamilies. E-cadherin is present on many types of epithelial cells and is especially important for embryonic development. N-cadherin is present on nerve, muscle, and lens cells and is also critical for embryonic development. P-cadherin is present on cells of the placenta and epidermis. Recent studies report that protocadherins are involved in a variety of cell-cell interactions (Suzuki, S.T. (1996) *J. Cell Sci.*

109:2609-2611). The intracellular anchorage of cadherins is regulated by their dynamic association with catenins, a family of cytoplasmic signal transduction proteins associated with the actin cytoskeleton. The anchorage of cadherins to the actin cytoskeleton appears to be regulated by protein tyrosine phosphorylation, and the cadherins are the target of phosphorylation-induced junctional
5 disassembly (Aberle, H. et al. (1996) *J. Cell. Biochem.* 61:514-523).

Integrins

Integrins are ubiquitous transmembrane adhesion molecules that link the ECM to the internal cytoskeleton. Integrins are composed of two noncovalently associated transmembrane glycoprotein subunits called α and β . Integrins function as receptors that play a role in signal transduction. For
10 example, binding of integrin to its extracellular ligand may stimulate changes in intracellular calcium levels or protein kinase activity (Sjaastad, M.D. and W.J. Nelson (1997) *BioEssays* 19:47-55). At least ten cell surface receptors of the integrin family recognize the ECM component fibronectin, which is involved in many different biological processes including cell migration and embryogenesis (Johansson, S. et al. (1997) *Front. Biosci.* 2:D126-D146).

Lectins

Lectins comprise a ubiquitous family of extracellular glycoproteins which bind cell surface carbohydrates specifically and reversibly, resulting in the agglutination of cells (reviewed in
Drickamer, K. and M.E. Taylor (1993) *Annu. Rev. Cell Biol.* 9:237-264). This function is particularly important for activation of the immune response. Lectins mediate the agglutination and
20 mitogenic stimulation of lymphocytes at sites of inflammation (Lasky, L.A. (1991) *J. Cell. Biochem.* 45:139-146; Paietta, E. et al. (1989) *J. Immunol.* 143:2850-2857).

Lectins are further classified into subfamilies based on carbohydrate-binding specificity and other criteria. The galectin subfamily, in particular, includes lectins that bind β -galactoside carbohydrate moieties in a thiol-dependent manner (reviewed in Hadari, Y.R. et al. (1998) *J. Biol.*
25 *Chem.* 270:3447-3453). Galectins are widely expressed and developmentally regulated. Because all galectins lack an N-terminal signal peptide, it is suggested that galectins are externalized through an atypical secretory mechanism. Two classes of galectins have been defined based on molecular weight and oligomerization properties. Small galectins form homodimers and are about 14 to 16 kilodaltons in mass, while large galectins are monomeric and about 29-37 kilodaltons.

30 Galectins contain a characteristic carbohydrate recognition domain (CRD). The CRD is about 140 amino acids and contains several stretches of about 1 - 10 amino acids which are highly conserved among all galectins. A particular 6-amino acid motif within the CRD contains conserved tryptophan and arginine residues which are critical for carbohydrate binding. The CRD of some galectins also contains cysteine residues which may be important for disulfide bond formation.

Secondary structure predictions indicate that the CRD forms several β -sheets.

Galectins play a number of roles in diseases and conditions associated with cell-cell and cell-matrix interactions. For example, certain galectins associate with sites of inflammation and bind to cell surface immunoglobulin E molecules. In addition, galectins may play an important role in cancer metastasis. Galectin overexpression is correlated with the metastatic potential of cancers in humans and mice. Moreover, anti-galectin antibodies inhibit processes associated with cell transformation, such as cell aggregation and anchorage-independent growth (See, for example, Su, Z.-Z. et al. (1996) Proc. Natl. Acad. Sci. USA 93:7252-7257).

Selectins

Selectins, or LEC-CAMs, comprise a specialized lectin subfamily involved primarily in inflammation and leukocyte adhesion (Reviewed in Lasky, supra). Selectins mediate the recruitment of leukocytes from the circulation to sites of acute inflammation and are expressed on the surface of vascular endothelial cells in response to cytokine signaling. Selectins bind to specific ligands on the leukocyte cell membrane and enable the leukocyte to adhere to and migrate along the endothelial surface. Binding of selectin to its ligand leads to polarized rearrangement of the actin cytoskeleton and stimulates signal transduction within the leukocyte (Brenner, B. et al. (1997) Biochem. Biophys. Res. Commun. 231:802-807; Hidari, K.I. et al. (1997) J. Biol. Chem. 272:28750-28756). Members of the selectin family possess three characteristic motifs: a lectin or carbohydrate recognition domain; an epidermal growth factor-like domain; and a variable number of short consensus repeats (scr or "sushi" repeats) which are also present in complement regulatory proteins. The selectins include lymphocyte adhesion molecule-1 (Lam-1 or L-selectin), endothelial leukocyte adhesion molecule-1 (ELAM-1 or E-selectin), and granule membrane protein-140 (GMP-140 or P-selectin) (Johnston, G.I. et al. (1989) Cell 56:1033-1044).

Antigen Recognition Molecules

All vertebrates have developed sophisticated and complex immune systems that provide protection from viral, bacterial, fungal, and parasitic infections. A key feature of the immune system is its ability to distinguish foreign molecules, or antigens, from "self" molecules. This ability is mediated primarily by secreted and transmembrane proteins expressed by leukocytes (white blood cells) such as lymphocytes, granulocytes, and monocytes. Most of these proteins belong to the immunoglobulin (Ig) superfamily, members of which contain one or more repeats of a conserved structural domain. This Ig domain is comprised of antiparallel β sheets joined by a disulfide bond in an arrangement called the Ig fold. Members of the Ig superfamily include T-cell receptors, major histocompatibility (MHC) proteins, antibodies, and immune cell-specific surface markers such as

CD4, CD8, and CD28.

MHC proteins are cell surface markers that bind to and present foreign antigens to T cells. MHC molecules are classified as either class I or class II. Class I MHC molecules (MHC I) are expressed on the surface of almost all cells and are involved in the presentation of antigen to cytotoxic T cells. For example, a cell infected with virus will degrade intracellular viral proteins and express the protein fragments bound to MHC I molecules on the cell surface. The MHC I/antigen complex is recognized by cytotoxic T-cells which destroy the infected cell and the virus within. Class II MHC molecules are expressed primarily on specialized antigen-presenting cells of the immune system, such as B-cells and macrophages. These cells ingest foreign proteins from the extracellular fluid and express MHC II/antigen complex on the cell surface. This complex activates helper T-cells, which then secrete cytokines and other factors that stimulate the immune response. MHC molecules also play an important role in organ rejection following transplantation. Rejection occurs when the recipient's T-cells respond to foreign MHC molecules on the transplanted organ in the same way as to self MHC molecules bound to foreign antigen. (Reviewed in Alberts, B. et al. (1994) Molecular Biology of the Cell, Garland Publishing, New York NY, pp. 1229-1246.)

Antibodies, or immunoglobulins, are either expressed on the surface of B-cells or secreted by B-cells into the circulation. Antibodies bind and neutralize foreign antigens in the blood and other extracellular fluids. The prototypical antibody is a tetramer consisting of two identical heavy polypeptide chains (H-chains) and two identical light polypeptide chains (L-chains) interlinked by disulfide bonds. This arrangement confers the characteristic Y-shape to antibody molecules. Antibodies are classified based on their H-chain composition. The five antibody classes, IgA, IgD, IgE, IgG and IgM, are defined by the α , δ , ϵ , γ , and μ H-chain types. There are two types of L-chains, κ and λ , either of which may associate as a pair with any H-chain pair. IgG, the most common class of antibody found in the circulation, is tetrameric, while the other classes of antibodies are generally variants or multimers of this basic structure.

H-chains and L-chains each contain an N-terminal variable region and a C-terminal constant region. The constant region consists of about 110 amino acids in L-chains and about 330 or 440 amino acids in H-chains. The amino acid sequence of the constant region is nearly identical among H- or L-chains of a particular class. The variable region consists of about 110 amino acids in both H- and L-chains. However, the amino acid sequence of the variable region differs among H- or L-chains of a particular class. Within each H- or L-chain variable region are three hypervariable regions of extensive sequence diversity, each consisting of about 5 to 10 amino acids. In the antibody molecule, the H- and L-chain hypervariable regions come together to form the antigen recognition site. (Reviewed in Alberts, supra, pp. 1206-1213 and 1216-1217.)

Both H-chains and L-chains contain repeated Ig domains. For example, a typical H-chain contains four Ig domains, three of which occur within the constant region and one of which occurs within the variable region and contributes to the formation of the antigen recognition site. Likewise, a typical L-chain contains two Ig domains, one of which occurs within the constant region and one of which occurs within the variable region.

The immune system is capable of recognizing and responding to any foreign molecule that enters the body. Therefore, the immune system must be armed with a full repertoire of antibodies against all potential antigens. Such antibody diversity is generated by somatic rearrangement of gene segments encoding variable and constant regions. These gene segments are joined together by site-specific recombination which occurs between highly conserved DNA sequences that flank each gene segment. Because there are hundreds of different gene segments, millions of unique genes can be generated combinatorially. In addition, imprecise joining of these segments and an unusually high rate of somatic mutation within these segments further contribute to the generation of a diverse antibody population.

T-cell receptors are both structurally and functionally related to antibodies. (Reviewed in Alberts, *supra*, pp. 1228-1229.) T-cell receptors are cell surface proteins that bind foreign antigens and mediate diverse aspects of the immune response. A typical T-cell receptor is a heterodimer comprised of two disulfide-linked polypeptide chains called α and β . Each chain is about 280 amino acids in length and contains one variable region and one constant region. Each variable or constant region folds into an Ig domain. The variable regions from the α and β chains come together in the heterodimer to form the antigen recognition site. T-cell receptor diversity is generated by somatic rearrangement of gene segments encoding the α and β chains. T-cell receptors recognize small peptide antigens that are expressed on the surface of antigen-presenting cells and pathogen-infected cells. These peptide antigens are presented on the cell surface in association with major histocompatibility proteins which provide the proper context for antigen recognition.

Secreted and Extracellular Matrix Molecules

Protein secretion is essential for cellular function. Protein secretion is mediated by a signal peptide located at the amino terminus of the protein to be secreted. The signal peptide is comprised of about ten to twenty hydrophobic amino acids which target the nascent protein from the ribosome to the endoplasmic reticulum (ER). Proteins targeted to the ER may either proceed through the secretory pathway or remain in any of the secretory organelles such as the ER, Golgi apparatus, or lysosomes. Proteins that transit through the secretory pathway are either secreted into the extracellular space or retained in the plasma membrane. Secreted proteins are often synthesized as inactive precursors that

are activated by post-translational processing events during transit through the secretory pathway. Such events include glycosylation, proteolysis, and removal of the signal peptide by a signal peptidase. Other events that may occur during protein transport include chaperone-dependent unfolding and folding of the nascent protein and interaction of the protein with a receptor or pore complex. Examples
5 of secreted proteins with amino terminal signal peptides include receptors, extracellular matrix molecules, cytokines, hormones, growth and differentiation factors, neuropeptides, vasomediators, ion channels, transporters/pumps, and proteases. (Reviewed in Alberts, B. et al. (1994) Molecular Biology of The Cell, Garland Publishing, New York NY, pp. 557-560, 582-592.)

The extracellular matrix (ECM) is a complex network of glycoproteins, polysaccharides,
10 proteoglycans, and other macromolecules that are secreted from the cell into the extracellular space. The ECM remains in close association with the cell surface and provides a supportive meshwork that profoundly influences cell shape, motility, strength, flexibility, and adhesion. In fact, adhesion of a cell to its surrounding matrix is required for cell survival except in the case of metastatic tumor cells, which have overcome the need for cell-ECM anchorage. This phenomenon suggests that the ECM
15 plays a critical role in the molecular mechanisms of growth control and metastasis. (Reviewed in Ruoslahti, E. (1996) *Sci. Am.* 275:72-77.) Furthermore, the ECM determines the structure and physical properties of connective tissue and is particularly important for morphogenesis and other processes associated with embryonic development and pattern formation.

The collagens comprise a family of ECM proteins that provide structure to bone, teeth, skin,
20 ligaments, tendons, cartilage, blood vessels, and basement membranes. Multiple collagen proteins have been identified. Three collagen molecules fold together in a triple helix stabilized by interchain disulfide bonds. Bundles of these triple helices then associate to form fibrils. Collagen primary structure consists of hundreds of (Gly-X-Y) repeats where about a third of the X and Y residues are Pro. Glycines are crucial to helix formation as the bulkier amino acid sidechains cannot fold into the triple
25 helical conformation. Because of these strict sequence requirements, mutations in collagen genes have severe consequences. Osteogenesis imperfecta patients have brittle bones that fracture easily; in severe cases patients die in utero or at birth. Ehlers-Danlos syndrome patients have hyperelastic skin, hypermobile joints, and susceptibility to aortic and intestinal rupture. Chondrodysplasia patients have short stature and ocular disorders. Alport syndrome patients have hematuria, sensorineural deafness,
30 and eye lens deformation. (Isselbacher, K.J. et al. (1994) Harrison's Principles of Internal Medicine, McGraw-Hill, Inc., New York NY, pp. 2105-2117; and Creighton, T.E. (1984) Proteins, Structures and Molecular Principles, W.H. Freeman and Company, New York NY, pp. 191-197.)

Elastin and related proteins confer elasticity to tissues such as skin, blood vessels, and lungs. Elastin is a highly hydrophobic protein of about 750 amino acids that is rich in proline and glycine

residues. Elastin molecules are highly cross-linked, forming an extensive extracellular network of fibers and sheets. Elastin fibers are surrounded by a sheath of microfibrils which are composed of a number of glycoproteins, including fibrillin. Mutations in the gene encoding fibrillin are responsible for Marfan's syndrome, a genetic disorder characterized by defects in connective tissue. In severe cases, the aortas of afflicted individuals are prone to rupture. (Reviewed in Alberts, *supra*, pp. 984-986.)

Fibronectin is a large ECM glycoprotein found in all vertebrates. Fibronectin exists as a dimer of two subunits, each containing about 2,500 amino acids. Each subunit folds into a rod-like structure containing multiple domains. The domains each contain multiple repeated modules, the most common of which is the type III fibronectin repeat. The type III fibronectin repeat is about 90 amino acids in length and is also found in other ECM proteins and in some plasma membrane and cytoplasmic proteins. Furthermore, some type III fibronectin repeats contain a characteristic tripeptide consisting of Arginine-Glycine-Aspartic acid (RGD). The RGD sequence is recognized by the integrin family of cell surface receptors and is also found in other ECM proteins. Disruption of both copies of the gene encoding fibronectin causes early embryonic lethality in mice. The mutant embryos display extensive morphological defects, including defects in the formation of the notochord, somites, heart, blood vessels, neural tube, and extraembryonic structures. (Reviewed in Alberts, *supra*, pp. 986-987.)

Laminin is a major glycoprotein component of the basal lamina which underlies and supports epithelial cell sheets. Laminin is one of the first ECM proteins synthesized in the developing embryo. Laminin is an 850 kilodalton protein composed of three polypeptide chains joined in the shape of a cross by disulfide bonds. Laminin is especially important for angiogenesis and in particular, for guiding the formation of capillaries. (Reviewed in Alberts, *supra*, pp. 990-991.)

There are many other types of proteinaceous ECM components, most of which can be classified as proteoglycans. Proteoglycans are composed of unbranched polysaccharide chains (glycosaminoglycans) attached to protein cores. Common proteoglycans include aggrecan, betaglycan, decorin, perlecan, serglycin, and syndecan-1. Some of these molecules not only provide mechanical support, but also bind to extracellular signaling molecules, such as fibroblast growth factor and transforming growth factor β , suggesting a role for proteoglycans in cell-cell communication and cell growth. (Reviewed in Alberts, *supra*, pp. 973-978.) Likewise, the glycoproteins tenascin-C and tenascin-R are expressed in developing and lesioned neural tissue and provide stimulatory and anti-adhesive (inhibitory) properties, respectively, for axonal growth. (Faissner, A. (1997) Cell Tissue Res. 290:331-341.)

Cytoskeletal Molecules

The cytoskeleton is a cytoplasmic network of protein fibers that mediate cell shape, structure,

and movement. The cytoskeleton supports the cell membrane and forms tracks along which organelles and other elements move in the cytosol. The cytoskeleton is a dynamic structure that allows cells to adopt various shapes and to carry out directed movements. Major cytoskeletal fibers include the microtubules, the microfilaments, and the intermediate filaments. Motor proteins, including myosin, dynein, and kinesin, drive movement of or along the fibers. The motor protein dynamin drives the formation of membrane vesicles. Accessory or associated proteins modify the structure or activity of the fibers while cytoskeletal membrane anchors connect the fibers to the cell membrane.

Tubulins

Microtubules, cytoskeletal fibers with a diameter of about 24 nm, have multiple roles in the cell. Bundles of microtubules form cilia and flagella, which are whip-like extensions of the cell membrane that are necessary for sweeping materials across an epithelium and for swimming of sperm, respectively. Marginal bands of microtubules in red blood cells and platelets are important for these cells' pliability. Organelles, membrane vesicles, and proteins are transported in the cell along tracks of microtubules. For example, microtubules run through nerve cell axons, allowing bi-directional transport of materials and membrane vesicles between the cell body and the nerve terminal. Failure to supply the nerve terminal with these vesicles blocks the transmission of neural signals. Microtubules are also critical to chromosomal movement during cell division. Both stable and short-lived populations of microtubules exist in the cell.

Microtubules are polymers of GTP-binding tubulin protein subunits. Each subunit is a heterodimer of α - and β - tubulin, multiple isoforms of which exist. The hydrolysis of GTP is linked to the addition of tubulin subunits at the end of a microtubule. The subunits interact head to tail to form protofilaments; the protofilaments interact side to side to form a microtubule. A microtubule is polarized, one end ringed with α -tubulin and the other with β -tubulin, and the two ends differ in their rates of assembly. Generally, each microtubule is composed of 13 protofilaments although 11 or 15 protofilament-microtubules are sometimes found. Cilia and flagella contain doublet microtubules. Microtubules grow from specialized structures known as centrosomes or microtubule-organizing centers (MTOCs). MTOCs may contain one or two centrioles, which are pinwheel arrays of triplet microtubules. The basal body, the organizing center located at the base of a cilium or flagellum, contains one centriole. Gamma tubulin present in the MTOC is important for nucleating the polymerization of α - and β - tubulin heterodimers but does not polymerize into microtubules.

Microtubule-Associated Proteins

Microtubule-associated proteins (MAPs) have roles in the assembly and stabilization of microtubules. One major family of MAPs, assembly MAPs, can be identified in neurons as well as

non-neuronal cells. Assembly MAPs are responsible for cross-linking microtubules in the cytosol. These MAPs are organized into two domains: a basic microtubule-binding domain and an acidic projection domain. The projection domain is the binding site for membranes, intermediate filaments, or other microtubules. Based on sequence analysis, assembly MAPs can be further grouped into two types: Type I and Type II. Type I MAPs, which include MAP1A and MAP1B, are large, filamentous molecules that co-purify with microtubules and are abundantly expressed in brain and testes. Type I MAPs contain several repeats of a positively-charged amino acid sequence motif that binds and neutralizes negatively charged tubulin, leading to stabilization of microtubules. MAP1A and MAP1B are each derived from a single precursor polypeptide that is subsequently proteolytically processed to generate one heavy chain and one light chain.

Another light chain, LC3, is a 16.4 kDa molecule that binds MAP1A, MAP1B, and microtubules. It is suggested that LC3 is synthesized from a source other than the MAP1A or MAP1B transcripts, and that the expression of LC3 may be important in regulating the microtubule binding activity of MAP1A and MAP1B during cell proliferation (Mann, S.S. et al. (1994) J. Biol. Chem. 269:11492-11497).

Type II MAPs, which include MAP2a, MAP2b, MAP2c, MAP4, and Tau, are characterized by three to four copies of an 18-residue sequence in the microtubule-binding domain. MAP2a, MAP2b, and MAP2c are found only in dendrites, MAP4 is found in non-neuronal cells, and Tau is found in axons and dendrites of nerve cells. Alternative splicing of the Tau mRNA leads to the existence of multiple forms of Tau protein. Tau phosphorylation is altered in neurodegenerative disorders such as Alzheimer's disease, Pick's disease, progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia and Parkinsonism linked to chromosome 17. The altered Tau phosphorylation leads to a collapse of the microtubule network and the formation of intraneuronal Tau aggregates (Spillantini, M.G. and M. Goedert (1998) Trends Neurosci. 21:428-433).

The protein pericentrin is found in the MTOC and has a role in microtubule assembly.

Actins

Microfilaments, cytoskeletal filaments with a diameter of about 7-9 nm, are vital to cell locomotion, cell shape, cell adhesion, cell division, and muscle contraction. Assembly and disassembly of the microfilaments allow cells to change their morphology. Microfilaments are the polymerized form of actin, the most abundant intracellular protein in the eukaryotic cell. Human cells contain six isoforms of actin. The three α -actins are found in different kinds of muscle, nonmuscle β -actin and nonmuscle γ -actin are found in nonmuscle cells, and another γ -actin is found in intestinal smooth muscle cells. G-actin, the monomeric form of actin, polymerizes into polarized, helical F-actin filaments, accompanied by the hydrolysis of ATP to ADP. Actin filaments associate to form

bundles and networks, providing a framework to support the plasma membrane and determine cell shape. These bundles and networks are connected to the cell membrane. In muscle cells, thin filaments containing actin slide past thick filaments containing the motor protein myosin during contraction. A family of actin-related proteins exist that are not part of the actin cytoskeleton, but rather associate with microtubules and dynein.

Actin-Associated Proteins

Actin-associated proteins have roles in cross-linking, severing, and stabilization of actin filaments and in sequestering actin monomers. Several of the actin-associated proteins have multiple functions. Bundles and networks of actin filaments are held together by actin cross-linking proteins. These proteins have two actin-binding sites, one for each filament. Short cross-linking proteins promote bundle formation while longer, more flexible cross-linking proteins promote network formation. Calmodulin-like calcium-binding domains in actin cross-linking proteins allow calcium regulation of cross-linking. Group I cross-linking proteins have unique actin-binding domains and include the 30 kD protein, EF-1a, fascin, and scriuin. Group II cross-linking proteins have a 7,000-MW actin-binding domain and include villin and dematin. Group III cross-linking proteins have pairs of a 26,000-MW actin-binding domain and include fimbrin, spectrin, dystrophin, ABP 120, and filamin.

Severing proteins regulate the length of actin filaments by breaking them into short pieces or by blocking their ends. Severing proteins include gCAP39, severin (fragmin), gelsolin, and villin. Capping proteins can cap the ends of actin filaments, but cannot break filaments. Capping proteins include CapZ and tropomodulin. The proteins thymosin and profilin sequester actin monomers in the cytosol, allowing a pool of unpolymerized actin to exist. The actin-associated proteins tropomyosin, troponin, and caldesmon regulate muscle contraction in response to calcium.

Intermediate Filaments and Associated Proteins

Intermediate filaments (IFs) are cytoskeletal fibers with a diameter of about 10 nm, intermediate between that of microfilaments and microtubules. IFs serve structural roles in the cell, reinforcing cells and organizing cells into tissues. IFs are particularly abundant in epidermal cells and in neurons. IFs are extremely stable, and, in contrast to microfilaments and microtubules, do not function in cell motility.

Five types of IF proteins are known in mammals. Type I and Type II proteins are the acidic and basic keratins, respectively. Heterodimers of the acidic and basic keratins are the building blocks of keratin IFs. Keratins are abundant in soft epithelia such as skin and cornea, hard epithelia such as nails and hair, and in epithelia that line internal body cavities. Mutations in keratin genes lead to epithelial diseases including epidermolysis bullosa simplex, bullous congenital ichthyosiform

erythroderma (epidermolytic hyperkeratosis), non-epidermolytic and epidermolytic palmoplantar keratoderma, ichthyosis bullosa of Siemens, pachyonychia congenita, and white sponge nevus. Some of these diseases result in severe skin blistering. (See, e.g., Wawersik, M. et al. (1997) *J. Biol. Chem.* 272:32557-32565; and Corden L.D. and W.H. McLean (1996) *Exp. Dermatol.* 5:297-307.)

5 Type III IF proteins include desmin, glial fibrillary acidic protein, vimentin, and peripherin. Desmin filaments in muscle cells link myofibrils into bundles and stabilize sarcomeres in contracting muscle. Glial fibrillary acidic protein filaments are found in the glial cells that surround neurons and astrocytes. Vimentin filaments are found in blood vessel endothelial cells, some epithelial cells, and mesenchymal cells such as fibroblasts, and are commonly associated with microtubules. Vimentin
10 filaments may have roles in keeping the nucleus and other organelles in place in the cell. Type IV IFs include the neurofilaments and nestin. Neurofilaments, composed of three polypeptides NF-L, NF-M, and NF-H, are frequently associated with microtubules in axons. Neurofilaments are responsible for the radial growth and diameter of an axon, and ultimately for the speed of nerve impulse transmission. Changes in phosphorylation and metabolism of neurofilaments are observed in neurodegenerative
15 diseases including amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease (Julien, J.P. and W.E. Mushynski (1998) *Prog. Nucleic Acid Res. Mol. Biol.* 61:1-23). Type V IFs, the lamins, are found in the nucleus where they support the nuclear membrane.

IFs have a central α -helical rod region interrupted by short nonhelical linker segments. The rod region is bracketed, in most cases, by non-helical head and tail domains. The rod regions of
20 intermediate filament proteins associate to form a coiled-coil dimer. A highly ordered assembly process leads from the dimers to the IFs. Neither ATP nor GTP is needed for IF assembly, unlike that of microfilaments and microtubules.

IF-associated proteins (IFAPs) mediate the interactions of IFs with one another and with other cell structures. IFAPs cross-link IFs into a bundle, into a network, or to the plasma membrane, and
25 may cross-link IFs to the microfilament and microtubule cytoskeleton. Microtubules and IFs are in particular closely associated. IFAPs include BPAG1, plakoglobin, desmoplakin I, desmoplakin II, plectin, ankyrin, filaggrin, and lamin B receptor.

Cytoskeletal-Membrane Anchors

Cytoskeletal fibers are attached to the plasma membrane by specific proteins. These
30 attachments are important for maintaining cell shape and for muscle contraction. In erythrocytes, the spectrin-actin cytoskeleton is attached to cell membrane by three proteins, band 4.1, ankyrin, and adducin. Defects in this attachment result in abnormally shaped cells which are more rapidly degraded by the spleen, leading to anemia. In platelets, the spectrin-actin cytoskeleton is also linked to the membrane by ankyrin; a second actin network is anchored to the membrane by filamin. In

muscle cells the protein dystrophin links actin filaments to the plasma membrane; mutations in the dystrophin gene lead to Duchenne muscular dystrophy. In adherens junctions and adhesion plaques the peripheral membrane proteins α -actinin and vinculin attach actin filaments to the cell membrane.

IFs are also attached to membranes by cytoskeletal-membrane anchors. The nuclear lamina is
5 attached to the inner surface of the nuclear membrane by the lamin B receptor. Vimentin IFs are attached to the plasma membrane by ankyrin and plectin. Desmosome and hemidesmosome membrane junctions hold together epithelial cells of organs and skin. These membrane junctions allow shear forces to be distributed across the entire epithelial cell layer, thus providing strength and rigidity to the epithelium. IFs in epithelial cells are attached to the desmosome by plakoglobin and
10 desmoplakins. The proteins that link IFs to hemidesmosomes are not known. Desmin IFs surround the sarcomere in muscle and are linked to the plasma membrane by paranemin, synemin, and ankyrin.

Myosin-related Motor Proteins

Myosins are actin-activated ATPases, found in eukaryotic cells, that couple hydrolysis of ATP with motion. Myosin provides the motor function for muscle contraction and intracellular
15 movements such as phagocytosis and rearrangement of cell contents during mitotic cell division (cytokinesis). The contractile unit of skeletal muscle, termed the sarcomere, consists of highly ordered arrays of thin actin-containing filaments and thick myosin-containing filaments. Crossbridges form between the thick and thin filaments, and the ATP-dependent movement of myosin heads within the thick filaments pulls the thin filaments, shortening the sarcomere and thus the muscle fiber.

20 Myosins are composed of one or two heavy chains and associated light chains. Myosin heavy chains contain an amino-terminal motor or head domain, a neck that is the site of light-chain binding, and a carboxy-terminal tail domain. The tail domains may associate to form an α -helical coiled coil. Conventional myosins, such as those found in muscle tissue, are composed of two myosin heavy-chain subunits, each associated with two light-chain subunits that bind at the neck
25 region and play a regulatory role. Unconventional myosins, believed to function in intracellular motion, may contain either one or two heavy chains and associated light chains. There is evidence for about 25 myosin heavy chain genes in vertebrates, more than half of them unconventional.

Dynein-related Motor Proteins

Dyneins are (-) end-directed motor proteins which act on microtubules. Two classes of
30 dyneins, cytosolic and axonemal, have been identified. Cytosolic dyneins are responsible for translocation of materials along cytoplasmic microtubules, for example, transport from the nerve terminal to the cell body and transport of endocytic vesicles to lysosomes. Cytoplasmic dyneins are also reported to play a role in mitosis. Axonemal dyneins are responsible for the beating of flagella and cilia. Dynein on one microtubule doublet walks along the adjacent microtubule doublet. This sliding

force produces bending forces that cause the flagellum or cilium to beat. Dyneins have a native mass between 1000 and 2000 kDa and contain either two or three force-producing heads driven by the hydrolysis of ATP. The heads are linked via stalks to a basal domain which is composed of a highly variable number of accessory intermediate and light chains.

5 Kinesin-related Motor Proteins

Kinesins are (+) end-directed motor proteins which act on microtubules. The prototypical kinesin molecule is involved in the transport of membrane-bound vesicles and organelles. This function is particularly important for axonal transport in neurons. Kinesin is also important in all cell types for the transport of vesicles from the Golgi complex to the endoplasmic reticulum. This role is critical for
10 maintaining the identity and functionality of these secretory organelles.

Kinesins define a ubiquitous, conserved family of over 50 proteins that can be classified into at least 8 subfamilies based on primary amino acid sequence, domain structure, velocity of movement, and cellular function. (Reviewed in Moore, J.D. and S.A. Endow (1996) Bioessays 18:207-219; and Hoyt, A.M. (1994) Curr. Opin. Cell Biol. 6:63-68.) The prototypical kinesin molecule is a heterotetramer
15 comprised of two heavy polypeptide chains (KHCs) and two light polypeptide chains (KLCs). The KHC subunits are typically referred to as "kinesin." KHC is about 1000 amino acids in length, and KLC is about 550 amino acids in length. Two KHCs dimerize to form a rod-shaped molecule with three distinct regions of secondary structure. At one end of the molecule is a globular motor domain that functions in ATP hydrolysis and microtubule binding. Kinesin motor domains are highly conserved
20 and share over 70% identity. Beyond the motor domain is an α -helical coiled-coil region which mediates dimerization. At the other end of the molecule is a fan-shaped tail that associates with molecular cargo. The tail is formed by the interaction of the KHC C-termini with the two KLCs.

Members of the more divergent subfamilies of kinesins are called kinesin-related proteins (KRPs), many of which function during mitosis in eukaryotes (Hoyt, *supra*). Some KRPs are required
25 for assembly of the mitotic spindle. *In vivo* and *in vitro* analyses suggest that these KRPs exert force on microtubules that comprise the mitotic spindle, resulting in the separation of spindle poles. Phosphorylation of KRP is required for this activity. Failure to assemble the mitotic spindle results in abortive mitosis and chromosomal aneuploidy, the latter condition being characteristic of cancer cells. In addition, a unique KRP, centromere protein E, localizes to the kinetochore of human mitotic
30 chromosomes and may play a role in their segregation to opposite spindle poles.

Dynamamin-related Motor Proteins

Dynamamin is a large GTPase motor protein that functions as a "molecular pinchase," generating a mechanochemical force used to sever membranes. This activity is important in forming clathrin-coated vesicles from coated pits in endocytosis and in the biogenesis of synaptic vesicles in

neurons. Binding of dynamin to a membrane leads to dynamin's self-assembly into spirals that may act to constrict a flat membrane surface into a tubule. GTP hydrolysis induces a change in conformation of the dynamin polymer that pinches the membrane tubule, leading to severing of the membrane tubule and formation of a membrane vesicle. Release of GDP and inorganic phosphate leads to dynamin disassembly. Following disassembly the dynamin may either dissociate from the membrane or remain associated to the vesicle and be transported to another region of the cell. Three homologous dynamin genes have been discovered, in addition to several dynamin-related proteins. Conserved dynamin regions are the N-terminal GTP-binding domain, a central pleckstrin homology domain that binds membranes, a central coiled-coil region that may activate dynamin's GTPase activity, and a C-terminal proline-rich domain that contains several motifs that bind SH3 domains on other proteins. Some dynamin-related proteins do not contain the pleckstrin homology domain or the proline-rich domain. (See McNiven, M.A. (1998) *Cell* 94:151-154; Scaife, R.M. and R.L. Margolis (1997) *Cell. Signal.* 9:395-401.)

The cytoskeleton is reviewed in Lodish, H. et al. (1995) Molecular Cell Biology, Scientific American Books, New York NY.

Ribosomal Molecules

Ribosomal RNAs (rRNAs) are assembled, along with ribosomal proteins, into ribosomes, which are cytoplasmic particles that translate messenger RNA into polypeptides. The eukaryotic ribosome is composed of a 60S (large) subunit and a 40S (small) subunit, which together form the 80S ribosome. In addition to the 18S, 28S, 5S, and 5.8S rRNAs, the ribosome also contains more than fifty proteins. The ribosomal proteins have a prefix which denotes the subunit to which they belong, either L (large) or S (small). Ribosomal protein activities include binding rRNA and organizing the conformation of the junctions between rRNA helices (Woodson, S.A. and N.B. Leontis (1998) *Curr. Opin. Struct. Biol.* 8:294-300; Ramakrishnan, V. and S.W. White (1998) *Trends Biochem. Sci.* 23:208-212.) Three important sites are identified on the ribosome. The aminoacyl-tRNA site (A site) is where charged tRNAs (with the exception of the initiator-tRNA) bind on arrival at the ribosome. The peptidyl-tRNA site (P site) is where new peptide bonds are formed, as well as where the initiator tRNA binds. The exit site (E site) is where deacylated tRNAs bind prior to their release from the ribosome. (The ribosome is reviewed in Stryer, L. (1995) Biochemistry W.H. Freeman and Company, New York NY, pp. 888-908; and Lodish, H. et al. (1995) Molecular Cell Biology Scientific American Books, New York NY, pp. 119-138.)

Chromatin Molecules

The nuclear DNA of eukaryotes is organized into chromatin. Two types of chromatin are observed: euchromatin, some of which may be transcribed, and heterochromatin so densely packed that much of it is inaccessible to transcription. Chromatin packing thus serves to regulate protein expression in eukaryotes. Bacteria lack chromatin and the chromatin-packing level of gene regulation.

5 The fundamental unit of chromatin is the nucleosome of 200 DNA base pairs associated with two copies each of histones H2A, H2B, H3, and H4. Adjacent nucleosomes are linked by another class of histones, H1. Low molecular weight non-histone proteins called the high mobility group (HMG), associated with chromatin, may function in the unwinding of DNA and stabilization of single-stranded DNA. Chromodomain proteins function in compaction of chromatin into its transcriptionally
10 silent heterochromatin form.

During mitosis, all DNA is compacted into heterochromatin and transcription ceases. Transcription in interphase begins with the activation of a region of chromatin. Active chromatin is decondensed. Decondensation appears to be accompanied by changes in binding coefficient, phosphorylation and acetylation states of chromatin histones. HMG proteins HMG13 and HMG17
15 selectively bind activated chromatin. Topoisomerases remove superhelical tension on DNA. The activated region decondenses, allowing gene regulatory proteins and transcription factors to assemble on the DNA.

Patterns of chromatin structure can be stably inherited, producing heritable patterns of gene expression. In mammals, one of the two X chromosomes in each female cell is inactivated by
20 condensation to heterochromatin during zygote development. The inactive state of this chromosome is inherited, so that adult females are mosaics of clusters of paternal-X and maternal-X clonal cell groups. The condensed X chromosome is reactivated in meiosis.

Chromatin is associated with disorders of protein expression such as thalassemia, a genetic anemia resulting from the removal of the locus control region (LCR) required for decondensation of the
25 globin gene locus.

For a review of chromatin structure and function see Alberts, B. et al. (1994) Molecular Cell Biology, third edition, Garland Publishing, Inc., New York NY, pp. 351-354, 433-439.

Electron Transfer Associated Molecules

30 Electron carriers such as cytochromes accept electrons from NADH or FADH₂ and donate them to other electron carriers. Most electron-transferring proteins, except ubiquinone, are prosthetic groups such as flavins, heme, FeS clusters, and copper, bound to inner membrane proteins. Adrenodoxin, for example, is an FeS protein that forms a complex with NADPH:adrenodoxin reductase and cytochrome p450. Cytochromes contain a heme prosthetic group, a porphyrin ring

containing a tightly bound iron atom. Electron transfer reactions play a crucial role in cellular energy production.

Energy is produced by the oxidation of glucose and fatty acids. Glucose is initially converted to pyruvate in the cytoplasm. Fatty acids and pyruvate are transported to the mitochondria for
5 complete oxidation to CO_2 coupled by enzymes to the transport of electrons from NADH and FADH_2 to oxygen and to the synthesis of ATP (oxidative phosphorylation) from ADP and P_i .

Pyruvate is transported into the mitochondria and converted to acetyl-CoA for oxidation via the citric acid cycle, involving pyruvate dehydrogenase components, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase. Enzymes involved in the citric acid cycle include: citrate synthetase,
10 aconitases, isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase complex including transsuccinylases, succinyl CoA synthetase, succinate dehydrogenase, fumarases, and malate dehydrogenase. Acetyl CoA is oxidized to CO_2 with concomitant formation of NADH, FADH_2 , and GTP. In oxidative phosphorylation, the transfer of electrons from NADH and FADH_2 to oxygen by dehydrogenases is coupled to the synthesis of ATP from ADP and P_i by the F_0F_1 ATPase complex in
15 the mitochondrial inner membrane. Enzyme complexes responsible for electron transport and ATP synthesis include the F_0F_1 ATPase complex, ubiquinone(CoQ)-cytochrome c reductase, ubiquinone reductase, cytochrome b, cytochrome c_1 , FeS protein, and cytochrome c oxidase.

ATP synthesis requires membrane transport enzymes including the phosphate transporter and the ATP-ADP antiport protein. The ATP-binding cassette (ABC) superfamily has also been suggested
20 as belonging to the mitochondrial transport group (Hogue, D.L. et al. (1999) J. Mol. Biol. 285:379-389). Brown fat uncoupling protein dissipates oxidative energy as heat, and may be involved the fever response to infection and trauma (Cannon, B. et al. (1998) Ann. NY Acad. Sci. 856:171-187).

Mitochondria are oval-shaped organelles comprising an outer membrane, a tightly folded inner membrane, an intermembrane space between the outer and inner membranes, and a matrix
25 inside the inner membrane. The outer membrane contains many porin molecules that allow ions and charged molecules to enter the intermembrane space, while the inner membrane contains a variety of transport proteins that transfer only selected molecules. Mitochondria are the primary sites of energy production in cells.

Mitochondria contain a small amount of DNA. Human mitochondrial DNA encodes 13
30 proteins, 22 tRNAs, and 2 rRNAs. Mitochondrial-DNA encoded proteins include NADH-Q reductase, a cytochrome reductase subunit, cytochrome oxidase subunits, and ATP synthase subunits.

Electron-transfer reactions also occur outside the mitochondria in locations such as the endoplasmic reticulum, which plays a crucial role in lipid and protein biosynthesis. Cytochrome b5 is a central electron donor for various reductive reactions occurring on the cytoplasmic surface of liver

endoplasmic reticulum. Cytochrome b5 has been found in Golgi, plasma, endoplasmic reticulum (ER), and microbody membranes.

For a review of mitochondrial metabolism and regulation, see Lodish, H. et al. (1995) Molecular Cell Biology, Scientific American Books, New York NY, pp. 745-797 and Stryer (1995) Biochemistry, W.H. Freeman and Co., San Francisco CA, pp 529-558, 988-989.

The majority of mitochondrial proteins are encoded by nuclear genes, are synthesized on cytosolic ribosomes, and are imported into the mitochondria. Nuclear-encoded proteins which are destined for the mitochondrial matrix typically contain positively-charged amino terminal signal sequences. Import of these preproteins from the cytoplasm requires a multisubunit protein complex in the outer membrane known as the translocase of outer mitochondrial membrane (TOM; previously designated MOM; Pfanner, N. et al. (1996) Trends Biochem. Sci. 21:51-52) and at least three inner membrane proteins which comprise the translocase of inner mitochondrial membrane (TIM; previously designated MIM; Pfanner, *supra*). An inside-negative membrane potential across the inner mitochondrial membrane is also required for preprotein import. Preproteins are recognized by surface receptor components of the TOM complex and are translocated through a proteinaceous pore formed by other TOM components. Proteins targeted to the matrix are then recognized by the import machinery of the TIM complex. The import systems of the outer and inner membranes can function independently (Segui-Real, B. et al. (1993) EMBO J. 12:2211-2218).

Once precursor proteins are in the mitochondria, the leader peptide is cleaved by a signal peptidase to generate the mature protein. Most leader peptides are removed in a one step process by a protease termed mitochondrial processing peptidase (MPP) (Paces, V. et al. (1993) Proc. Natl. Acad. Sci. USA 90:5355-5358). In some cases a two-step process occurs in which MPP generates an intermediate precursor form which is cleaved by a second enzyme, mitochondrial intermediate peptidase, to generate the mature protein.

Mitochondrial dysfunction leads to impaired calcium buffering, generation of free radicals that may participate in deleterious intracellular and extracellular processes, changes in mitochondrial permeability and oxidative damage which is observed in several neurodegenerative diseases. Neurodegenerative diseases linked to mitochondrial dysfunction include some forms of Alzheimer's disease, Friedreich's ataxia, familial amyotrophic lateral sclerosis, and Huntington's disease (Beal, M.F. (1998) Biochim. Biophys. Acta 1366:211-213). The myocardium is heavily dependent on oxidative metabolism, so mitochondrial dysfunction often leads to heart disease (DiMauro, S. and M. Hirano (1998) Curr. Opin. Cardiol 13:190-197). Mitochondria are implicated in disorders of cell proliferation, since they play an important role in a cell's decision to proliferate or self-destruct through apoptosis. The oncoprotein Bcl-2, for example, promotes cell proliferation by stabilizing

mitochondrial membranes so that apoptosis signals are not released (Susin, S.A. (1998) *Biochim. Biophys. Acta* 1366:151-165).

Transcription Factor Molecules

5 Multicellular organisms are comprised of diverse cell types that differ dramatically both in structure and function. The identity of a cell is determined by its characteristic pattern of gene expression, and different cell types express overlapping but distinctive sets of genes throughout development. Spatial and temporal regulation of gene expression is critical for the control of cell proliferation, cell differentiation, apoptosis, and other processes that contribute to organismal
10 development. Furthermore, gene expression is regulated in response to extracellular signals that mediate cell-cell communication and coordinate the activities of different cell types. Appropriate gene regulation also ensures that cells function efficiently by expressing only those genes whose functions are required at a given time.

Transcriptional regulatory proteins are essential for the control of gene expression. Some of
15 these proteins function as transcription factors that initiate, activate, repress, or terminate gene transcription. Transcription factors generally bind to the promoter, enhancer, and upstream regulatory regions of a gene in a sequence-specific manner, although some factors bind regulatory elements within or downstream of a gene's coding region. Transcription factors may bind to a specific region of DNA singly or as a complex with other accessory factors. (Reviewed in Lewin, B. (1990) Genes
20 IV, Oxford University Press, New York NY, and Cell Press, Cambridge MA, pp. 554-570.)

The double helix structure and repeated sequences of DNA create topological and chemical features which can be recognized by transcription factors. These features are hydrogen bond donor and acceptor groups, hydrophobic patches, major and minor grooves, and regular, repeated stretches of sequence which induce distinct bends in the helix. Typically, transcription factors recognize
25 specific DNA sequence motifs of about 20 nucleotides in length. Multiple, adjacent transcription factor-binding motifs may be required for gene regulation.

Many transcription factors incorporate DNA-binding structural motifs which comprise either α helices or β sheets that bind to the major groove of DNA. Four well-characterized structural motifs are helix-turn-helix, zinc finger, leucine zipper, and helix-loop-helix. Proteins containing these
30 motifs may act alone as monomers, or they may form homo- or heterodimers that interact with DNA.

The helix-turn-helix motif consists of two α helices connected at a fixed angle by a short chain of amino acids. One of the helices binds to the major groove. Helix-turn-helix motifs are exemplified by the homeobox motif which is present in homeodomain proteins. These proteins are critical for specifying the anterior-posterior body axis during development and are conserved

throughout the animal kingdom. The Antennapedia and Ultrabithorax proteins of Drosophila melanogaster are prototypical homeodomain proteins (Pabo, C.O. and R.T. Sauer (1992) Annu. Rev. Biochem. 61:1053-1095).

The zinc finger motif, which binds zinc ions, generally contains tandem repeats of about 30 amino acids consisting of periodically spaced cysteine and histidine residues. Examples of this sequence pattern, designated C2H2 and C3HC4 ("RING" finger), have been described (Lewin, supra). Zinc finger proteins each contain an α helix and an antiparallel β sheet whose proximity and conformation are maintained by the zinc ion. Contact with DNA is made by the arginine preceding the α helix and by the second, third, and sixth residues of the α helix. Variants of the zinc finger motif include poorly defined cysteine-rich motifs which bind zinc or other metal ions. These motifs may not contain histidine residues and are generally nonrepetitive.

The leucine zipper motif comprises a stretch of amino acids rich in leucine which can form an amphipathic α helix. This structure provides the basis for dimerization of two leucine zipper proteins. The region adjacent to the leucine zipper is usually basic, and upon protein dimerization, is optimally positioned for binding to the major groove. Proteins containing such motifs are generally referred to as bZIP transcription factors.

The helix-loop-helix motif (HLH) consists of a short α helix connected by a loop to a longer α helix. The loop is flexible and allows the two helices to fold back against each other and to bind to DNA. The transcription factor Myc contains a prototypical HLH motif.

Most transcription factors contain characteristic DNA binding motifs, and variations on the above motifs and new motifs have been and are currently being characterized (Faisst, S. and S. Meyer (1992) Nucleic Acids Res. 20:3-26).

Many neoplastic disorders in humans can be attributed to inappropriate gene expression. Malignant cell growth may result from either excessive expression of tumor promoting genes or insufficient expression of tumor suppressor genes (Cleary, M.L. (1992) Cancer Surv. 15:89-104). Chromosomal translocations may also produce chimeric loci which fuse the coding sequence of one gene with the regulatory regions of a second unrelated gene. Such an arrangement likely results in inappropriate gene transcription, potentially contributing to malignancy.

In addition, the immune system responds to infection or trauma by activating a cascade of events that coordinate the progressive selection, amplification, and mobilization of cellular defense mechanisms. A complex and balanced program of gene activation and repression is involved in this process. However, hyperactivity of the immune system as a result of improper or insufficient regulation of gene expression may result in considerable tissue or organ damage. This damage is well documented in immunological responses associated with arthritis, allergens, heart attack, stroke, and

infections (Isselbacher, K.J. et al. (1996) Harrison's Principles of Internal Medicine, 13/e, McGraw Hill, Inc. and Teton Data Systems Software).

Furthermore, the generation of multicellular organisms is based upon the induction and coordination of cell differentiation at the appropriate stages of development. Central to this process is differential gene expression, which confers the distinct identities of cells and tissues throughout the body. Failure to regulate gene expression during development can result in developmental disorders. Human developmental disorders caused by mutations in zinc finger-type transcriptional regulators include: urogenital developmental abnormalities associated with WT1; Greig cephalopolysyndactyly, Pallister-Hall syndrome, and postaxial polydactyly type A (GLI3); and Townes-Brocks syndrome, characterized by anal, renal, limb, and ear abnormalities (SALL1) (Engelkamp, D. and V. van Heyningen (1996) *Curr. Opin. Genet. Dev.* 6:334-342; Kohlhasse, J. et al. (1999) *Am. J. Hum. Genet.* 64:435-445).

Cell Membrane Molecules

Eukaryotic cells are surrounded by plasma membranes which enclose the cell and maintain an environment inside the cell that is distinct from its surroundings. In addition, eukaryotic organisms are distinct from prokaryotes in possessing many intracellular organelle and vesicle structures. Many of the metabolic reactions which distinguish eukaryotic biochemistry from prokaryotic biochemistry take place within these structures. The plasma membrane and the membranes surrounding organelles and vesicles are composed of phosphoglycerides, fatty acids, cholesterol, phospholipids, glycolipids, proteoglycans, and proteins. These components confer identity and functionality to the membranes with which they associate.

Integral Membrane Proteins

The majority of known integral membrane proteins are transmembrane proteins (TM) which are characterized by an extracellular, a transmembrane, and an intracellular domain. TM domains are typically comprised of 15 to 25 hydrophobic amino acids which are predicted to adopt an α -helical conformation. TM proteins are classified as bitopic (Types I and II) and polytopic (Types III and IV) (Singer, S.J. (1990) *Annu. Rev. Cell Biol.* 6:247-296). Bitopic proteins span the membrane once while polytopic proteins contain multiple membrane-spanning segments. TM proteins function as cell-surface receptors, receptor-interacting proteins, transporters of ions or metabolites, ion channels, cell anchoring proteins, and cell type-specific surface antigens.

Many membrane proteins (MPs) contain amino acid sequence motifs that target these proteins to specific subcellular sites. Examples of these motifs include PDZ domains, KDEL, RGD, NGR, and GSL sequence motifs, von Willebrand factor A (vWFA) domains, and EGF-like domains. RGD,

NGR, and GSL motif-containing peptides have been used as drug delivery agents in targeted cancer treatment of tumor vasculature (Arap, W. et al. (1998) Science 279:377-380). Furthermore, MPs may also contain amino acid sequence motifs, such as the carbohydrate recognition domain (CRD), that mediate interactions with extracellular or intracellular molecules.

5 G-Protein Coupled Receptors

G-protein coupled receptors (GPCR) are a superfamily of integral membrane proteins which transduce extracellular signals. GPCRs include receptors for biogenic amines, lipid mediators of inflammation, peptide hormones, and sensory signal mediators. The structure of these highly-conserved receptors consists of seven hydrophobic transmembrane regions, an extracellular
10 N-terminus, and a cytoplasmic C-terminus. Three extracellular loops alternate with three intracellular loops to link the seven transmembrane regions. Cysteine disulfide bridges connect the second and third extracellular loops. The most conserved regions of GPCRs are the transmembrane regions and the first two cytoplasmic loops. A conserved, acidic-Arg-aromatic residue triplet present in the second cytoplasmic loop may interact with G proteins. A GPCR consensus pattern is characteristic of
15 most proteins belonging to this superfamily (ExPASy PROSITE document PS00237; and Watson, S. and S. Arkinstall (1994) The G-protein Linked Receptor Facts Book, Academic Press, San Diego CA, pp. 2-6). Mutations and changes in transcriptional activation of GPCR-encoding genes have been associated with neurological disorders such as schizophrenia, Parkinson's disease, Alzheimer's disease, drug addiction, and feeding disorders.

20 Scavenger Receptors

Macrophage scavenger receptors with broad ligand specificity may participate in the binding of low density lipoproteins (LDL) and foreign antigens. Scavenger receptors types I and II are trimeric membrane proteins with each subunit containing a small N-terminal intracellular domain, a transmembrane domain, a large extracellular domain, and a C-terminal cysteine-rich domain. The
25 extracellular domain contains a short spacer region, an α -helical coiled-coil region, and a triple helical collagen-like region. These receptors have been shown to bind a spectrum of ligands, including chemically modified lipoproteins and albumin, polyribonucleotides, polysaccharides, phospholipids, and asbestos (Matsumoto, A. et al. (1990) Proc. Natl. Acad. Sci. USA 87:9133-9137; and Elomaa, O. et al. (1995) Cell 80:603-609). The scavenger receptors are thought to play a key role in
30 atherogenesis by mediating uptake of modified LDL in arterial walls, and in host defense by binding bacterial endotoxins, bacteria, and protozoa.

Tetraspan Family Proteins

The transmembrane 4 superfamily (TM4SF) or tetraspan family is a multigene family encoding type III integral membrane proteins (Wright, M.D. and M.G. Tomlinson (1994) Immunol.

Today 15:588-594). The TM4SF is comprised of membrane proteins which traverse the cell membrane four times. Members of the TM4SF include platelet and endothelial cell membrane proteins, melanoma-associated antigens, leukocyte surface glycoproteins, colonal carcinoma antigens, tumor-associated antigens, and surface proteins of the schistosome parasites (Jankowski, S.A. (1994) Oncogene 9:1205-1211). Members of the TM4SF share about 25-30% amino acid sequence identity with one another.

A number of TM4SF members have been implicated in signal transduction, control of cell adhesion, regulation of cell growth and proliferation, including development and oncogenesis, and cell motility, including tumor cell metastasis. Expression of TM4SF proteins is associated with a variety of tumors and the level of expression may be altered when cells are growing or activated.

Tumor Antigens

Tumor antigens are cell surface molecules that are differentially expressed in tumor cells relative to normal cells. Tumor antigens distinguish tumor cells immunologically from normal cells and provide diagnostic and therapeutic targets for human cancers (Takagi, S. et al. (1995) Int. J. Cancer 61:706-715; Liu, E. et al. (1992) Oncogene 7:1027-1032).

Leukocyte Antigens

Other types of cell surface antigens include those identified on leukocytic cells of the immune system. These antigens have been identified using systematic, monoclonal antibody (mAb)-based "shot gun" techniques. These techniques have resulted in the production of hundreds of mAbs directed against unknown cell surface leukocytic antigens. These antigens have been grouped into "clusters of differentiation" based on common immunocytochemical localization patterns in various differentiated and undifferentiated leukocytic cell types. Antigens in a given cluster are presumed to identify a single cell surface protein and are assigned a "cluster of differentiation" or "CD" designation. Some of the genes encoding proteins identified by CD antigens have been cloned and verified by standard molecular biology techniques. CD antigens have been characterized as both transmembrane proteins and cell surface proteins anchored to the plasma membrane via covalent attachment to fatty acid-containing glycolipids such as glycosylphosphatidylinositol (GPI). (Reviewed in Barclay, A.N. et al. (1995) The Leucocyte Antigen Facts Book, Academic Press, San Diego CA, pp. 17-20.)

Ion Channels

Ion channels are found in the plasma membranes of virtually every cell in the body. For example, chloride channels mediate a variety of cellular functions including regulation of membrane potentials and absorption and secretion of ions across epithelial membranes. Chloride channels also regulate the pH of organelles such as the Golgi apparatus and endosomes (see, e.g., Greger, R. (1988)

Annu. Rev. Physiol. 50:111-122). Electrophysiological and pharmacological properties of chloride channels, including ion conductance, current-voltage relationships, and sensitivity to modulators, suggest that different chloride channels exist in muscles, neurons, fibroblasts, epithelial cells, and lymphocytes.

- 5 Many ion channels have sites for phosphorylation by one or more protein kinases including protein kinase A, protein kinase C, tyrosine kinase, and casein kinase II, all of which regulate ion channel activity in cells. Inappropriate phosphorylation of proteins in cells has been linked to changes in cell cycle progression and cell differentiation. Changes in the cell cycle have been linked to induction of apoptosis or cancer. Changes in cell differentiation have been linked to diseases and
10 disorders of the reproductive system, immune system, skeletal muscle, and other organ systems.

Proton Pumps

- Proton ATPases comprise a large class of membrane proteins that use the energy of ATP hydrolysis to generate an electrochemical proton gradient across a membrane. The resultant gradient may be used to transport other ions across the membrane (Na^+ , K^+ , or Cl^-) or to maintain organelle
15 pH. Proton ATPases are further subdivided into the mitochondrial F-ATPases, the plasma membrane ATPases, and the vacuolar ATPases. The vacuolar ATPases establish and maintain an acidic pH within various organelles involved in the processes of endocytosis and exocytosis (Mellman, I. et al. (1986) Annu. Rev. Biochem. 55:663-700).

- Proton-coupled, 12 membrane-spanning domain transporters such as PEPT 1 and PEPT 2 are
20 responsible for gastrointestinal absorption and for renal reabsorption of peptides using an electrochemical H^+ gradient as the driving force. Another type of peptide transporter, the TAP transporter, is a heterodimer consisting of TAP 1 and TAP 2 and is associated with antigen processing. Peptide antigens are transported across the membrane of the endoplasmic reticulum by TAP so they can be expressed on the cell surface in association with MHC molecules. Each TAP
25 protein consists of multiple hydrophobic membrane spanning segments and a highly conserved ATP-binding cassette (Boll, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:284-289). Pathogenic microorganisms, such as herpes simplex virus, may encode inhibitors of TAP-mediated peptide transport in order to evade immune surveillance (Marusina, K. and J.J Manaco (1996) Curr. Opin. Hematol. 3:19-26).

ABC Transporters

The ATP-binding cassette (ABC) transporters, also called the "traffic ATPases", comprise a superfamily of membrane proteins that mediate transport and channel functions in prokaryotes and eukaryotes (Higgins, C.F. (1992) Annu. Rev. Cell Biol. 8:67-113). ABC proteins share a similar overall structure and significant sequence homology. All ABC proteins contain a conserved domain

of approximately two hundred amino acid residues which includes one or more nucleotide binding domains. Mutations in ABC transporter genes are associated with various disorders, such as hyperbilirubinemia II/Dubin-Johnson syndrome, recessive Stargardt's disease, X-linked adrenoleukodystrophy, multidrug resistance, celiac disease, and cystic fibrosis.

5 Peripheral and Anchored Membrane Proteins

Some membrane proteins are not membrane-spanning but are attached to the plasma membrane via membrane anchors or interactions with integral membrane proteins. Membrane anchors are covalently joined to a protein post-translationally and include such moieties as prenyl, myristyl, and glycosylphosphatidyl inositol groups. Membrane localization of peripheral and
10 anchored proteins is important for their function in processes such as receptor-mediated signal transduction. For example, prenylation of Ras is required for its localization to the plasma membrane and for its normal and oncogenic functions in signal transduction.

Vesicle Coat Proteins

Intercellular communication is essential for the development and survival of multicellular
15 organisms. Cells communicate with one another through the secretion and uptake of protein signaling molecules. The uptake of proteins into the cell is achieved by the endocytic pathway, in which the interaction of extracellular signaling molecules with plasma membrane receptors results in the formation of plasma membrane-derived vesicles that enclose and transport the molecules into the cytosol. These transport vesicles fuse with and mature into endosomal and lysosomal (digestive)
20 compartments. The secretion of proteins from the cell is achieved by exocytosis, in which molecules inside of the cell proceed through the secretory pathway. In this pathway, molecules transit from the ER to the Golgi apparatus and finally to the plasma membrane, where they are secreted from the cell.

Several steps in the transit of material along the secretory and endocytic pathways require the formation of transport vesicles. Specifically, vesicles form at the transitional endoplasmic reticulum
25 (tER), the rim of Golgi cisternae, the face of the Trans-Golgi Network (TGN), the plasma membrane (PM), and tubular extensions of the endosomes. Vesicle formation occurs when a region of membrane buds off from the donor organelle. The membrane-bound vesicle contains proteins to be transported and is surrounded by a proteinaceous coat, the components of which are recruited from the cytosol. Two different classes of coat protein have been identified. Clathrin coats form on
30 vesicles derived from the TGN and PM, whereas coatamer (COP) coats form on vesicles derived from the ER and Golgi. COP coats can be further classified as COPI, involved in retrograde traffic through the Golgi and from the Golgi to the ER, and COPII, involved in anterograde traffic from the ER to the Golgi (Mellman, *supra*).

In clathrin-based vesicle formation, adapter proteins bring vesicle cargo and coat proteins

together at the surface of the budding membrane. Adapter protein-1 and -2 select cargo from the TGN and plasma membrane, respectively, based on molecular information encoded on the cytoplasmic tail of integral membrane cargo proteins. Adapter proteins also recruit clathrin to the bud site. Clathrin is a protein complex consisting of three large and three small polypeptide chains
5 arranged in a three-legged structure called a triskelion. Multiple triskelions and other coat proteins appear to self-assemble on the membrane to form a coated pit. This assembly process may serve to deform the membrane into a budding vesicle. GTP-bound ADP-ribosylation factor (Arf) is also incorporated into the coated assembly. Another small G-protein, dynamin, forms a ring complex around the neck of the forming vesicle and may provide the mechanochemical force to seal the bud,
10 thereby releasing the vesicle. The coated vesicle complex is then transported through the cytosol. During the transport process, Arf-bound GTP is hydrolyzed to GDP, and the coat dissociates from the transport vesicle (West, M.A. et al. (1997) *J. Cell Biol.* 138:1239-1254).

Vesicles which bud from the ER and the Golgi are covered with a protein coat similar to the clathrin coat of endocytic and TGN vesicles. The coat protein (COP) is assembled from cytosolic
15 precursor molecules at specific budding regions on the organelle. The COP coat consists of two major components, a G-protein (Arf or Sar) and coat protomer (coatomer). Coatomer is an equimolar complex of seven proteins, termed alpha-, beta-, beta'-, gamma-, delta-, epsilon- and zeta-COP. The coatomer complex binds to dilysine motifs contained on the cytoplasmic tails of integral membrane proteins. These include the KKXX retrieval motif of membrane proteins of the ER and
20 dibasic/diphenylamine motifs of members of the p24 family. The p24 family of type I membrane proteins represent the major membrane proteins of COPI vesicles (Harter, C. and F.T. Wieland (1998) *Proc. Natl. Acad. Sci. USA* 95:11649-11654).

Organelle Associated Molecules

25 Eukaryotic cells are organized into various cellular organelles which has the effect of separating specific molecules and their functions from one another and from the cytosol. Within the cell, various membrane structures surround and define these organelles while allowing them to interact with one another and the cell environment through both active and passive transport processes. Important cell organelles include the nucleus, the Golgi apparatus, the endoplasmic
30 reticulum, mitochondria, peroxisomes, lysosomes, endosomes, and secretory vesicles.

Nucleus

The cell nucleus contains all of the genetic information of the cell in the form of DNA, and the components and machinery necessary for replication of DNA and for transcription of DNA into RNA. (See Alberts, B. et al. (1994) Molecular Biology of the Cell, Garland Publishing Inc., New

York NY, pp. 335-399.) DNA is organized into compact structures in the nucleus by interactions with various DNA-binding proteins such as histones and non-histone chromosomal proteins. DNA-specific nucleases, DNases, partially degrade these compacted structures prior to DNA replication or transcription. DNA replication takes place with the aid of DNA helicases which
5 unwind the double-stranded DNA helix, and DNA polymerases that duplicate the separated DNA strands.

Transcriptional regulatory proteins are essential for the control of gene expression. Some of these proteins function as transcription factors that initiate, activate, repress, or terminate gene transcription. Transcription factors generally bind to the promoter, enhancer, and upstream
10 regulatory regions of a gene in a sequence-specific manner, although some factors bind regulatory elements within or downstream of a gene's coding region. Transcription factors may bind to a specific region of DNA singly or as a complex with other accessory factors. (Reviewed in Lewin, B. (1990) Genes IV, Oxford University Press, New York NY, and Cell Press, Cambridge MA, pp. 554-570.) Many transcription factors incorporate DNA-binding structural motifs which comprise either α
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20 insufficient expression of tumor suppressor genes (Cleary, M.L. (1992) *Cancer Surv.* 15:89-104). Chromosomal translocations may also produce chimeric loci which fuse the coding sequence of one gene with the regulatory regions of a second unrelated gene. Such an arrangement likely results in inappropriate gene transcription, potentially contributing to malignancy.

In addition, the immune system responds to infection or trauma by activating a cascade of
25 events that coordinate the progressive selection, amplification, and mobilization of cellular defense mechanisms. A complex and balanced program of gene activation and repression is involved in this process. However, hyperactivity of the immune system as a result of improper or insufficient regulation of gene expression may result in considerable tissue or organ damage. This damage is well documented in immunological responses associated with arthritis, allergens, heart attack, stroke, and
30 infections (Isselbacher, K.J. et al. (1996) Harrison's Principles of Internal Medicine, 13/e, McGraw Hill, Inc. and Teton Data Systems Software).

Transcription of DNA into RNA also takes place in the nucleus catalyzed by RNA polymerases. Three types of RNA polymerase exist. RNA polymerase I makes large ribosomal RNAs, while RNA polymerase III makes a variety of small, stable RNAs including 5S ribosomal

RNA and the transfer RNAs (tRNA). RNA polymerase II transcribes genes that will be translated into proteins. The primary transcript of RNA polymerase II is called heterogenous nuclear RNA (hnRNA), and must be further processed by splicing to remove non-coding sequences called introns. RNA splicing is mediated by small nuclear ribonucleoprotein complexes, or snRNPs, producing
5 mature messenger RNA (mRNA) which is then transported out of the nucleus for translation into proteins.

Nucleolus

The nucleolus is a highly organized subcompartment in the nucleus that contains high concentrations of RNA and proteins and functions mainly in ribosomal RNA synthesis and assembly
10 (Alberts, et al. supra, pp. 379-382). Ribosomal RNA (rRNA) is a structural RNA that is complexed with proteins to form ribonucleoprotein structures called ribosomes. Ribosomes provide the platform on which protein synthesis takes place.

Ribosomes are assembled in the nucleolus initially from a large, 45S rRNA combined with a variety of proteins imported from the cytoplasm, as well as smaller, 5S rRNAs. Later processing of
15 the immature ribosome results in formation of smaller ribosomal subunits which are transported from the nucleolus to the cytoplasm where they are assembled into functional ribosomes.

Endoplasmic Reticulum

In eukaryotes, proteins are synthesized within the endoplasmic reticulum (ER), delivered from the ER to the Golgi apparatus for post-translational processing and sorting, and transported from the
20 Golgi to specific intracellular and extracellular destinations. Synthesis of integral membrane proteins, secreted proteins, and proteins destined for the lumen of a particular organelle occurs on the rough endoplasmic reticulum (ER). The rough ER is so named because of the rough appearance in electron micrographs imparted by the attached ribosomes on which protein synthesis proceeds. Synthesis of proteins destined for the ER actually begins in the cytosol with the synthesis of a specific signal
25 peptide which directs the growing polypeptide and its attached ribosome to the ER membrane where the signal peptide is removed and protein synthesis is completed. Soluble proteins destined for the ER lumen, for secretion, or for transport to the lumen of other organelles pass completely into the ER lumen. Transmembrane proteins destined for the ER or for other cell membranes are translocated across the ER membrane but remain anchored in the lipid bilayer of the membrane by one or more
30 membrane-spanning α -helical regions.

Translocated polypeptide chains destined for other organelles or for secretion also fold and assemble in the ER lumen with the aid of certain "resident" ER proteins. Protein folding in the ER is aided by two principal types of protein isomerases, protein disulfide isomerase (PDI), and peptidyl-prolyl isomerase (PPI). PDI catalyzes the oxidation of free sulfhydryl groups in cysteine residues to

form intramolecular disulfide bonds in proteins. PPI, an enzyme that catalyzes the isomerization of certain proline imide bonds in oligopeptides and proteins, is considered to govern one of the rate limiting steps in the folding of many proteins to their final functional conformation. The cyclophilins represent a major class of PPI that was originally identified as the major receptor for the immunosuppressive drug cyclosporin A (Handschumacher, R.E. et al. (1984) Science 226:544-547). Molecular "chaperones" such as BiP (binding protein) in the ER recognize incorrectly folded proteins as well as proteins not yet folded into their final form and bind to them, both to prevent improper aggregation between them, and to promote proper folding.

The "N-linked" glycosylation of most soluble secreted and membrane-bound proteins by oligosacchrides linked to asparagine residues in proteins is also performed in the ER. This reaction is catalyzed by a membrane-bound enzyme, oligosaccharyl transferase.

Golgi Apparatus

The Golgi apparatus is a complex structure that lies adjacent to the ER in eukaryotic cells and serves primarily as a sorting and dispatching station for products of the ER (Alberts, et al. *supra*, pp. 600-610). Additional posttranslational processing, principally additional glycosylation, also occurs in the Golgi. Indeed, the Golgi is a major site of carbohydrate synthesis, including most of the glycosaminoglycans of the extracellular matrix. N-linked oligosaccharides, added to proteins in the ER, are also further modified in the Golgi by the addition of more sugar residues to form complex N-linked oligosaccharides. "O-linked" glycosylation of proteins also occurs in the Golgi by the addition of N-acetylgalactosamine to the hydroxyl group of a serine or threonine residue followed by the sequential addition of other sugar residues to the first. This process is catalyzed by a series of glycosyltransferases each specific for a particular donor sugar nucleotide and acceptor molecule (Lodish, H. et al. (1995) *Molecular Cell Biology*, W.H. Freeman and Co., New York NY, pp.700-708). In many cases; both N- and O-linked oligosaccharides appear to be required for the secretion of proteins or the movement of plasma membrane glycoproteins to the cell surface.

The terminal compartment of the Golgi is the Trans-Golgi Network (TGN), where both membrane and luminal proteins are sorted for their final destination. Transport (or secretory) vesicles destined for intracellular compartments, such as lysosomes, bud off of the TGN. Other transport vesicles bud off containing proteins destined for the plasma membrane, such as receptors, adhesion molecules, and ion channels, and secretory proteins, such as hormones, neurotransmitters, and digestive enzymes.

Vacuoles

The vacuole system is a collection of membrane bound compartments in eukaryotic cells that functions in the processes of endocytosis and exocytosis. They include phagosomes, lysosomes,

endosomes, and secretory vesicles. Endocytosis is the process in cells of internalizing nutrients, solutes or small particles (pinocytosis) or large particles such as internalized receptors, viruses, bacteria, or bacterial toxins (phagocytosis). Exocytosis is the process of transporting molecules to the cell surface. It facilitates placement or localization of membrane-bound receptors or other membrane proteins and secretion of hormones, neurotransmitters, digestive enzymes, wastes, etc.

A common property of all of these vacuoles is an acidic pH environment ranging from approximately pH 4.5-5.0. This acidity is maintained by the presence of a proton ATPase that uses the energy of ATP hydrolysis to generate an electrochemical proton gradient across a membrane (Mellman, I. et al. (1986) *Annu. Rev. Biochem.* 55:663-700). Eukaryotic vacuolar proton ATPase (vp-ATPase) is a multimeric enzyme composed of 3-10 different subunits. One of these subunits is a highly hydrophobic polypeptide of approximately 16 kDa that is similar to the proteolipid component of vp-ATPases from eubacteria, fungi, and plant vacuoles (Mandel, M. et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:5521-5524). The 16 kDa proteolipid component is the major subunit of the membrane portion of vp-ATPase and functions in the transport of protons across the membrane.

Lysosomes

Lysosomes are membranous vesicles containing various hydrolytic enzymes used for the controlled intracellular digestion of macromolecules. Lysosomes contain some 40 types of enzymes including proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases, all of which are acid hydrolases that function at a pH of about 5. Lysosomes are surrounded by a unique membrane containing transport proteins that allow the final products of macromolecule degradation, such as sugars, amino acids, and nucleotides, to be transported to the cytosol where they may be either excreted or reutilized by the cell. A vp-ATPase, such as that described above, maintains the acidic environment necessary for hydrolytic activity (Alberts, *supra*, pp. 610-611).

Endosomes

Endosomes are another type of acidic vacuole that is used to transport substances from the cell surface to the interior of the cell in the process of endocytosis. Like lysosomes, endosomes have an acidic environment provided by a vp-ATPase (Alberts et al. *supra*, pp. 610-618). Two types of endosomes are apparent based on tracer uptake studies that distinguish their time of formation in the cell and their cellular location. Early endosomes are found near the plasma membrane and appear to function primarily in the recycling of internalized receptors back to the cell surface. Late endosomes appear later in the endocytic process close to the Golgi apparatus and the nucleus, and appear to be associated with delivery of endocytosed material to lysosomes or to the TGN where they may be recycled. Specific proteins are associated with particular transport vesicles and their target compartments that may provide selectivity in targeting vesicles to their proper compartments. A

cytosolic prenylated GTP-binding protein, Rab, is one such protein. Rabs 4, 5, and 11 are associated with the early endosome, whereas Rabs 7 and 9 associate with the late endosome.

Mitochondria

Mitochondria are oval-shaped organelles comprising an outer membrane, a tightly folded
5 inner membrane, an intermembrane space between the outer and inner membranes, and a matrix inside the inner membrane. The outer membrane contains many porin molecules that allow ions and charged molecules to enter the intermembrane space, while the inner membrane contains a variety of transport proteins that transfer only selected molecules. Mitochondria are the primary sites of energy production in cells.

10 Energy is produced by the oxidation of glucose and fatty acids. Glucose is initially converted to pyruvate in the cytoplasm. Fatty acids and pyruvate are transported to the mitochondria for complete oxidation to CO_2 coupled by enzymes to the transport of electrons from NADH and FADH_2 to oxygen and to the synthesis of ATP (oxidative phosphorylation) from ADP and P_i .

Pyruvate is transported into the mitochondria and converted to acetyl-CoA for oxidation via
15 the citric acid cycle, involving pyruvate dehydrogenase components, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase. Enzymes involved in the citric acid cycle include: citrate synthetase, aconitases, isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase complex including transsuccinylases, succinyl CoA synthetase, succinate dehydrogenase, fumarases, and malate dehydrogenase. Acetyl CoA is oxidized to CO_2 with concomitant formation of NADH, FADH_2 , and
20 GTP. In oxidative phosphorylation, the transfer of electrons from NADH and FADH_2 to oxygen by dehydrogenases is coupled to the synthesis of ATP from ADP and P_i by the F_0F_1 ATPase complex in the mitochondrial inner membrane. Enzyme complexes responsible for electron transport and ATP synthesis include the F_0F_1 ATPase complex, ubiquinone(CoQ)-cytochrome c reductase, ubiquinone reductase, cytochrome b, cytochrome c_1 , FeS protein, and cytochrome c oxidase.

Peroxisomes

25 Peroxisomes, like mitochondria, are a major site of oxygen utilization. They contain one or more enzymes, such as catalase and urate oxidase, that use molecular oxygen to remove hydrogen atoms from specific organic substrates in an oxidative reaction that produces hydrogen peroxide (Alberts, *supra*, pp. 574-577). Catalase oxidizes a variety of substrates including phenols, formic
30 acid, formaldehyde, and alcohol and is important in peroxisomes of liver and kidney cells for detoxifying various toxic molecules that enter the bloodstream. Another major function of oxidative reactions in peroxisomes is the breakdown of fatty acids in a process called β oxidation. β oxidation results in shortening of the alkyl chain of fatty acids by blocks of two carbon atoms that are converted to acetyl CoA and exported to the cytosol for reuse in biosynthetic reactions.

Also like mitochondria, peroxisomes import their proteins from the cytosol using a specific signal sequence located near the C-terminus of the protein. The importance of this import process is evident in the inherited human disease Zellweger syndrome, in which a defect in importing proteins into peroxisomes leads to a peroxisomal deficiency resulting in severe abnormalities in the brain, liver, and kidneys, and death soon after birth. One form of this disease has been shown to be due to a mutation in the gene encoding a peroxisomal integral membrane protein called peroxisome assembly factor-1.

The discovery of new human molecules satisfies a need in the art by providing new compositions which are useful in the diagnosis, study, prevention, and treatment of diseases associated with, as well as effects of exogenous compounds on, the expression of human molecules.

SUMMARY OF THE INVENTION

The present invention relates to nucleic acid sequences comprising human diagnostic and therapeutic polynucleotides (dithp) as presented in the Sequence Listing. The dithp uniquely identify genes encoding human structural, functional, and regulatory molecules.

The invention provides an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). In one alternative, the polynucleotide comprises a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211. In another alternative, the polynucleotide comprises at least 60 contiguous nucleotides of a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). The invention further provides a composition for the detection of expression of human diagnostic and therapeutic polynucleotides comprising at least one isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d);

and a detectable label.

The invention also provides a method for detecting a target polynucleotide in a sample, said target polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). The method comprises a) amplifying said target polynucleotide or a fragment thereof using polymerase chain reaction amplification, and b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

The invention also provides a method for detecting a target polynucleotide in a sample, said target polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). The method comprises a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide, and b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof. In one alternative, the probe comprises at least 30 contiguous nucleotides. In another alternative, the probe comprises at least 60 contiguous nucleotides.

The invention further provides a recombinant polynucleotide comprising a promoter sequence operably linked to an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide. In a further alternative, the invention provides a method for producing a human diagnostic and therapeutic polypeptide, the method comprising a) culturing a cell under conditions suitable for expression of the human diagnostic and therapeutic polypeptide,

wherein said cell is transformed with the recombinant polynucleotide, and b) recovering the human diagnostic and therapeutic polypeptide so expressed.

The invention also provides a purified human diagnostic and therapeutic polypeptide (DITHP) encoded by at least one polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211. Additionally, the invention provides an isolated antibody which specifically binds to the human diagnostic and therapeutic polypeptide. The invention further provides a method of identifying a test compound which specifically binds to the human diagnostic and therapeutic polypeptide, the method comprising the steps of a) providing a test compound; b) combining the human diagnostic and therapeutic polypeptide with the test compound for a sufficient time and under suitable conditions for binding; and c) detecting binding of the human diagnostic and therapeutic polypeptide to the test compound, thereby identifying the test compound which specifically binds the human diagnostic and therapeutic polypeptide.

The invention further provides a microarray wherein at least one element of the microarray is an isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). The invention also provides a method for generating a transcript image of a sample which contains polynucleotides. The method comprises a) labeling the polynucleotides of the sample, b) contacting the elements of the microarray with the labeled polynucleotides of the sample under conditions suitable for the formation of a hybridization complex, and c) quantifying the expression of the polynucleotides in the sample.

Additionally, the invention provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; c) a polynucleotide sequence complementary to a); d) a polynucleotide sequence complementary to b); and e) an RNA equivalent of a) through d). The method comprises a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide, and c) comparing the expression of the target polynucleotide in the presence of varying amounts of the compound and in the absence of the compound.

The invention further provides a method for assessing toxicity of a test compound, said method

comprising a) treating a biological sample containing nucleic acids with the test compound; b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of i) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; ii) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; iii) a polynucleotide sequence complementary to i), iv) a polynucleotide sequence complementary to ii), and v) an RNA equivalent of i)-iv). Hybridization occurs under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence selected from the group consisting of i) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; ii) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211; iii) a polynucleotide sequence complementary to i), iv) a polynucleotide sequence complementary to ii), and v) an RNA equivalent of i)-iv), and alternatively, the target polynucleotide comprises a fragment of a polynucleotide sequence selected from the group consisting of i-v above; c) quantifying the amount of hybridization complex; and d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

The invention further provides an isolated polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:212-422, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:212-422, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:212-422, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:212-422. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:212-422.

DESCRIPTION OF THE TABLES

Table 1 shows the sequence identification numbers (SEQ ID NO:s) and template identification numbers (template IDs) corresponding to the polynucleotides of the present invention, along with their GenBank hits (GI Numbers), probability scores, and functional annotations corresponding to the GenBank hits.

Table 2 shows the sequence identification numbers (SEQ ID NO:s) and template identification

numbers (template IDs) corresponding to the polynucleotides of the present invention, along with polynucleotide segments of each template sequence as defined by the indicated "start" and "stop" nucleotide positions. The reading frames of the polynucleotide segments and the Pfam hits, Pfam descriptions, and E-values corresponding to the polypeptide domains encoded by the polynucleotide segments are indicated.

Table 3 shows the sequence identification numbers (SEQ ID NO:s) and template identification numbers (template IDs) corresponding to the polynucleotides of the present invention, along with polynucleotide segments of each template sequence as defined by the indicated "start" and "stop" nucleotide positions. The reading frames of the polynucleotide segments are shown, and the polypeptides encoded by the polynucleotide segments constitute either signal peptide (SP) or transmembrane (TM) domains, as indicated. The membrane topology of the encoded polypeptide sequence is indicated, the N-terminus (N) listed as being oriented to either the cytosolic (in) or non-cytosolic (out) side of the cell membrane or organelle.

Table 4 shows the sequence identification numbers (SEQ ID NO:s) corresponding to the polynucleotides of the present invention, along with component sequence identification numbers (component IDs) corresponding to each template. The component sequences, which were used to assemble the template sequences, are defined by the indicated "start" and "stop" nucleotide positions along each template.

Table 5 shows the tissue distribution profiles for the templates of the invention.

Table 6 shows the sequence identification numbers (SEQ ID NO:s) corresponding to the polypeptides of the present invention, along with the reading frames used to obtain the polypeptide segments, the lengths of the polypeptide segments, the "start" and "stop" nucleotide positions of the polynucleotide sequences used to define the encoded polypeptide segments, the GenBank hits (GI Numbers), probability scores, and functional annotations corresponding to the GenBank hits.

Table 7 summarizes the bioinformatics tools which are useful for analysis of the polynucleotides of the present invention. The first column of Table 7 lists analytical tools, programs, and algorithms, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences).

DETAILED DESCRIPTION OF THE INVENTION

Before the nucleic acid sequences and methods are presented, it is to be understood that this

invention is not limited to the particular machines, methods, and materials described. Although particular embodiments are described, machines, methods, and materials similar or equivalent to these embodiments may be used to practice the invention. The preferred machines, methods, and materials set forth are not intended to limit the scope of the invention which is limited only by the appended
5 claims.

The singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. All technical and scientific terms have the meanings commonly understood by one of ordinary skill in the art. All publications are incorporated by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are presented and which might be used in
10 connection with the invention. Nothing in the specification is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Definitions

As used herein, the lower case "dithp" refers to a nucleic acid sequence, while the upper case
15 "DITHP" refers to an amino acid sequence encoded by dithp. A "full-length" dithp refers to a nucleic acid sequence containing the entire coding region of a gene endogenously expressed in human tissue.

"Adjuvants" are materials such as Freund's adjuvant, mineral gels (aluminum hydroxide), and surface active substances (lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol) which may be administered to increase a host's immunological
20 response.

"Allele" refers to an alternative form of a nucleic acid sequence. Alleles result from a "mutation," a change or an alternative reading of the genetic code. Any given gene may have none, one, or many allelic forms. Mutations which give rise to alleles include deletions, additions, or substitutions of nucleotides. Each of these changes may occur alone, or in combination with the others, one or more
25 times in a given nucleic acid sequence. The present invention encompasses allelic dithp.

"Amino acid sequence" refers to a peptide, a polypeptide, or a protein of either natural or synthetic origin. The amino acid sequence is not limited to the complete, endogenous amino acid sequence and may be a fragment, epitope, variant, or derivative of a protein expressed by a nucleic acid sequence.

30 "Amplification" refers to the production of additional copies of a sequence and is carried out using polymerase chain reaction (PCR) technologies well known in the art.

"Antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')₂, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind DITHP polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of

interest as the immunizing antigen. The polypeptide or peptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

“Antisense sequence” refers to a sequence capable of specifically hybridizing to a target sequence. The antisense sequence may include DNA, RNA, or any nucleic acid mimic or analog such as peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates, methylphosphonates, or benzylphosphonates; oligonucleotides having modified sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine.

“Antisense sequence” refers to a sequence capable of specifically hybridizing to a target sequence. The antisense sequence can be DNA, RNA, or any nucleic acid mimic or analog.

“Antisense technology” refers to any technology which relies on the specific hybridization of an antisense sequence to a target sequence.

A “bin” is a portion of computer memory space used by a computer program for storage of data, and bounded in such a manner that data stored in a bin may be retrieved by the program.

“Biologically active” refers to an amino acid sequence having a structural, regulatory, or biochemical function of a naturally occurring amino acid sequence.

“Clone joining” is a process for combining gene bins based upon the bins' containing sequence information from the same clone. The sequences may assemble into a primary gene transcript as well as one or more splice variants.

“Complementary” describes the relationship between two single-stranded nucleic acid sequences that anneal by base-pairing (5'-A-G-T-3' pairs with its complement 3'-T-C-A-5').

A “component sequence” is a nucleic acid sequence selected by a computer program such as PHRED and used to assemble a consensus or template sequence from one or more component sequences.

A “consensus sequence” or “template sequence” is a nucleic acid sequence which has been assembled from overlapping sequences, using a computer program for fragment assembly such as the GELVIEW fragment assembly system (Genetics Computer Group (GCG), Madison WI) or using a relational database management system (RDMS).

“Conservative amino acid substitutions” are those substitutions that, when made, least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids

which may be substituted for an original amino acid in a protein and which are regarded as conservative substitutions.

	Original Residue	Conservative Substitution
5	Ala	Gly, Ser
	Arg	His, Lys
	Asn	Asp, Gln, His
	Asp	Asn, Glu
	Cys	Ala, Ser
10	Gln	Asn, Glu, His
	Glu	Asp, Gln, His
	Gly	Ala
	His	Asn, Arg, Gln, Glu
	Ile	Leu, Val
15	Leu	Ile, Val
	Lys	Arg, Gln, Glu
	Met	Leu, Ile
	Phe	His, Met, Leu, Trp, Tyr
	Ser	Cys, Thr
20	Thr	Ser, Val
	Trp	Phe, Tyr
	Tyr	His, Phe, Trp
	Val	Ile, Leu, Thr

25

Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

“Deletion” refers to a change in either a nucleic or amino acid sequence in which at least one
30 nucleotide or amino acid residue, respectively, is absent.

“Derivative” refers to the chemical modification of a nucleic acid sequence, such as by replacement of hydrogen by an alkyl, acyl, amino, hydroxyl, or other group.

The terms “element” and “array element” refer to a polynucleotide, polypeptide, or other chemical compound having a unique and defined position on a microarray.

35 “E-value” refers to the statistical probability that a match between two sequences occurred by chance.

A “fragment” is a unique portion of dithp or DITHP which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise
40 from 10 to 1000 contiguous amino acid residues or nucleotides. A fragment used as a probe, primer,

antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous amino acid residues or nucleotides in length.

Fragments may be preferentially selected from certain regions of a molecule. For example, a

polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first

5 250 or 500 amino acids (or first 25% or 50%) of a polypeptide as shown in a certain defined sequence.

Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing and the figures, may be encompassed by the present embodiments.

A fragment of dithp comprises a region of unique polynucleotide sequence that specifically identifies dithp, for example, as distinct from any other sequence in the same genome. A fragment of

10 dithp is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish dithp from related polynucleotide sequences. The precise length of a fragment of dithp and the region of dithp to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of DITHP is encoded by a fragment of dithp. A fragment of DITHP comprises a

15 region of unique amino acid sequence that specifically identifies DITHP. For example, a fragment of

DITHP is useful as an immunogenic peptide for the development of antibodies that specifically

recognize DITHP. The precise length of a fragment of DITHP and the region of DITHP to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

20 A "full length" nucleotide sequence is one containing at least a start site for translation to a protein sequence, followed by an open reading frame and a stop site, and encoding a "full length" polypeptide.

"Hit" refers to a sequence whose annotation will be used to describe a given template. Criteria for selecting the top hit are as follows: if the template has one or more exact nucleic acid matches, the

25 top hit is the exact match with highest percent identity. If the template has no exact matches but has significant protein hits, the top hit is the protein hit with the lowest E-value. If the template has no significant protein hits, but does have significant non-exact nucleotide hits, the top hit is the nucleotide hit with the lowest E-value.

"Homology" refers to sequence similarity either between a reference nucleic acid sequence and
30 at least a fragment of a dithp or between a reference amino acid sequence and a fragment of a DITHP.

"Hybridization" refers to the process by which a strand of nucleotides anneals with a complementary strand through base pairing. Specific hybridization is an indication that two nucleic acid sequences share a high degree of identity. Specific hybridization complexes form under defined annealing conditions, and remain hybridized after the "washing" step. The defined hybridization

conditions include the annealing conditions and the washing step(s), the latter of which is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid probes that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely
5 determinable and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency.

Generally, stringency of hybridization is expressed with reference to the temperature under which the wash step is carried out. Generally, such wash temperatures are selected to be about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and
10 pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating T_m and conditions for nucleic acid hybridization is well known and can be found in Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

15 High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, or 55°C may be used. SSC concentration may be varied from about 0.2 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, denatured
20 salmon sperm DNA at about 100-200 µg/ml. Useful variations on these conditions will be readily apparent to those skilled in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their resultant proteins.

Other parameters, such as temperature, salt concentration, and detergent concentration may be
25 varied to achieve the desired stringency. Denaturants, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as RNA:DNA hybridizations. Appropriate hybridization conditions are routinely determinable by one of ordinary skill in the art.

"Immunogenic" describes the potential for a natural, recombinant, or synthetic peptide, epitope, polypeptide, or protein to induce antibody production in appropriate animals, cells, or cell lines.

30 "Insertion" or "addition" refers to a change in either a nucleic or amino acid sequence in which at least one nucleotide or residue, respectively, is added to the sequence.

"Labeling" refers to the covalent or noncovalent joining of a polynucleotide, polypeptide, or antibody with a reporter molecule capable of producing a detectable or measurable signal.

"Microarray" is any arrangement of nucleic acids, amino acids, antibodies, etc., on a substrate. The substrate may be a solid support such as beads, glass, paper, nitrocellulose, nylon, or an appropriate membrane.

"Linkers" are short stretches of nucleotide sequence which may be added to a vector or a dithp
5 to create restriction endonuclease sites to facilitate cloning. "Polylinkers" are engineered to incorporate multiple restriction enzyme sites and to provide for the use of enzymes which leave 5' or 3' overhangs (e.g., BamHI, EcoRI, and HindIII) and those which provide blunt ends (e.g., EcoRV, SnaBI, and StuI).

"Naturally occurring" refers to an endogenous polynucleotide or polypeptide that may be isolated from viruses or prokaryotic or eukaryotic cells.

10 "Nucleic acid sequence" refers to the specific order of nucleotides joined by phosphodiester bonds in a linear, polymeric arrangement. Depending on the number of nucleotides, the nucleic acid sequence can be considered an oligomer, oligonucleotide, or polynucleotide. The nucleic acid can be DNA, RNA, or any nucleic acid analog, such as PNA, may be of genomic or synthetic origin, may be either double-stranded or single-stranded, and can represent either the sense or antisense
15 (complementary) strand.

"Oligomer" refers to a nucleic acid sequence of at least about 6 nucleotides and as many as about 60 nucleotides, preferably about 15 to 40 nucleotides, and most preferably between about 20 and 30 nucleotides, that may be used in hybridization or amplification technologies. Oligomers may be used as, e.g., primers for PCR, and are usually chemically synthesized.

20 "Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

25 "Peptide nucleic acid" (PNA) refers to a DNA mimic in which nucleotide bases are attached to a pseudopeptide backbone to increase stability. PNAs, also designated antigene agents, can prevent gene expression by targeting complementary messenger RNA.

The phrases "percent identity" and "% identity", as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a
30 standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence

alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and Sharp, P.M. (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191.

For pairwise alignments of polynucleotide sequences, the default parameters are set as follows:

- 5 Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequence pairs.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search
10 Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at <http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to determine alignment between a known polynucleotide sequence and other sequences on a variety of databases. Also available is a tool called "BLAST 2
15 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bl2/>. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version
20 2.0.9 (May-07-1999) set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Reward for match: 1

Penalty for mismatch: -2

Open Gap: 5 and Extension Gap: 2 penalties

25 *Gap x drop-off: 50*

Expect: 10

Word Size: 11

Filter: on

Percent identity may be measured over the length of an entire defined sequence, for example, as
30 defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences

shown herein, in figures or Sequence Listings, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in
5 nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity", as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment
10 methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the hydrophobicity and acidity of the substituted residue, thus preserving the structure (and therefore function) of the folded polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment
15 program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

20 Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) with blastp set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Open Gap: 11 and Extension Gap: 1 penalty

25 *Gap x drop-off: 50*

Expect: 10

Word Size: 3

Filter: on

Percent identity may be measured over the length of an entire defined polypeptide sequence, for
30 example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length

supported by the sequences shown herein, in figures or Sequence Listings, may be used to describe a length over which percentage identity may be measured.

“Post-translational modification” of a DITHP may involve lipidation, glycosylation, phosphorylation, acetylation, racemization, proteolytic cleavage, and other modifications known in the art. These processes may occur synthetically or biochemically. Biochemical modifications will vary by cell type depending on the enzymatic milieu and the DITHP.

“Probe” refers to dithp or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. “Primers” are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the figures and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel et al., 1987, Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis et al., 1990, PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection

program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from
5 their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and
10 polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

15 "Purified" refers to molecules, either polynucleotides or polypeptides that are isolated or separated from their natural environment and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other compounds with which they are naturally associated.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence.
20 This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence.
25 Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be use to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

"Regulatory element" refers to a nucleic acid sequence from nontranslated regions of a gene,
30 and includes enhancers, promoters, introns, and 3' untranslated regions, which interact with host proteins to carry out or regulate transcription or translation.

"Reporter" molecules are chemical or biochemical moieties used for labeling a nucleic acid, an amino acid, or an antibody. They include radionuclides; enzymes; fluorescent, chemiluminescent, or

chromogenic agents; substrates; cofactors; inhibitors; magnetic particles; and other moieties known in the art.

An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the
5 nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

"Sample" is used in its broadest sense. Samples may contain nucleic or amino acids, antibodies, or other materials, and may be derived from any source (e.g., bodily fluids including, but not limited to, saliva, blood, and urine; chromosome(s), organelles, or membranes isolated from a cell;
10 genomic DNA, RNA, or cDNA in solution or bound to a substrate; and cleared cells or tissues or blots or imprints from such cells or tissues).

"Specific binding" or "specifically binding" refers to the interaction between a protein or peptide and its agonist, antibody, antagonist, or other binding partner. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope,
15 recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

"Substitution" refers to the replacement of at least one nucleotide or amino acid by a different
20 nucleotide or amino acid.

"Substrate" refers to any suitable rigid or semi-rigid support including, e.g., membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles or capillaries. The substrate can have a variety of surface forms, such as wells,
trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

25 A "transcript image" refers to the collective pattern of gene expression by a particular tissue or cell type under given conditions at a given time.

"Transformation" refers to a process by which exogenous DNA enters a recipient cell. Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid
30 sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed.

"Transformants" include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as cells which transiently express inserted DNA or RNA.

A "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or *in vitro* fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, and plants and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989), *supra*.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 25% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 30%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or even at least 98% or greater sequence identity over a certain defined length. The variant may result in "conservative" amino acid changes which do not affect structural and/or chemical properties. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

In an alternative, variants of the polynucleotides of the present invention may be generated through recombinant methods. One possible method is a DNA shuffling technique such as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) Nat. Biotechnol. 17:793-797; Christians, F.C. et al. (1999) Nat.

Biotechnol. 17:259-264; and Crameri, A. et al. (1996) Nat. Biotechnol. 14:315-319) to alter or improve the biological properties of DITHP, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to
5 selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial" breeding and rapid molecular evolution. For example, fragments of a single gene containing random point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively,
10 fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable manner.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of
15 the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

20 THE INVENTION

In a particular embodiment, cDNA sequences derived from human tissues and cell lines were aligned based on nucleotide sequence identity and assembled into "consensus" or "template" sequences which are designated by the template identification numbers (template IDs) in column 2 of Table 1. The sequence identification numbers (SEQ ID NO:s) corresponding to the template IDs are shown in
25 column 1. The template sequences have similarity to GenBank sequences, or "hits," as designated by the GI Numbers in column 3. The statistical probability of each GenBank hit is indicated by a probability score in column 4, and the functional annotation corresponding to each GenBank hit is listed in column 5.

The invention incorporates the nucleic acid sequences of these templates as disclosed in the
30 Sequence Listing and the use of these sequences in the diagnosis and treatment of disease states characterized by defects in human molecules. The invention further utilizes these sequences in hybridization and amplification technologies, and in particular, in technologies which assess gene expression patterns correlated with specific cells or tissues and their responses in vivo or in vitro to

pharmaceutical agents, toxins, and other treatments. In this manner, the sequences of the present invention are used to develop a transcript image for a particular cell or tissue.

Derivation of Nucleic Acid Sequences

5 cDNA was isolated from libraries constructed using RNA derived from normal and diseased human tissues and cell lines. The human tissues and cell lines used for cDNA library construction were selected from a broad range of sources to provide a diverse population of cDNAs representative of gene transcription throughout the human body. Descriptions of the human tissues and cell lines used for cDNA library construction are provided in the LIFESEQ database (Incyte Genomics, Inc. (Incyte), Palo
10 Alto CA). Human tissues were broadly selected from, for example, cardiovascular, dermatologic, endocrine, gastrointestinal, hematopoietic/immune system, musculoskeletal, neural, reproductive, and urologic sources.

Cell lines used for cDNA library construction were derived from, for example, leukemic cells, teratocarcinomas, neuroepitheliomas, cervical carcinoma, lung fibroblasts, and endothelial cells. Such
15 cell lines include, for example, THP-1, Jurkat, HUVEC, hNT2, WI38, HeLa, and other cell lines commonly used and available from public depositories (American Type Culture Collection, Manassas VA). Prior to mRNA isolation, cell lines were untreated, treated with a pharmaceutical agent such as 5'-aza-2'-deoxycytidine, treated with an activating agent such as lipopolysaccharide in the case of leukocytic cell lines, or, in the case of endothelial cell lines, subjected to shear stress.

20

Sequencing of the cDNAs

Methods for DNA sequencing are well known in the art. Conventional enzymatic methods employ the Klenow fragment of DNA polymerase I, SEQUENASE DNA polymerase (U.S. Biochemical Corporation, Cleveland OH), Taq polymerase (Applied Biosystems, Foster City CA),
25 thermostable T7 polymerase (Amersham Pharmacia Biotech, Inc. (Amersham Pharmacia Biotech), Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies Inc. (Life Technologies), Gaithersburg MD), to extend the nucleic acid sequence from an oligonucleotide primer annealed to the DNA template of interest. Methods have been developed for the use of both single-stranded and double-stranded
30 templates. Chain termination reaction products may be electrophoresed on urea-polyacrylamide gels and detected either by autoradiography (for radioisotope-labeled nucleotides) or by fluorescence (for fluorophore-labeled nucleotides). Automated methods for mechanized reaction preparation, sequencing, and analysis using fluorescence detection methods have been developed. Machines used to prepare cDNAs for sequencing can include the MICROLAB 2200 liquid transfer system (Hamilton Company

(Hamilton), Reno NV), Peltier thermal cycler (PTC200; MJ Research, Inc. (MJ Research), Watertown MA), and ABI CATALYST 800 thermal cycler (Applied Biosystems). Sequencing can be carried out using, for example, the ABI 373 or 377 (Applied Biosystems) or MEGABACE 1000 (Molecular Dynamics, Inc. (Molecular Dynamics), Sunnyvale CA) DNA sequencing systems, or other automated and manual sequencing systems well known in the art.

The nucleotide sequences of the Sequence Listing have been prepared by current, state-of-the-art, automated methods and, as such, may contain occasional sequencing errors or unidentified nucleotides. Such unidentified nucleotides are designated by an N. These infrequent unidentified bases do not represent a hindrance to practicing the invention for those skilled in the art. Several methods employing standard recombinant techniques may be used to correct errors and complete the missing sequence information. (See, e.g., those described in Ausubel, F.M. et al. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY; and Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY.)

Assembly of cDNA Sequences

Human polynucleotide sequences may be assembled using programs or algorithms well known in the art. Sequences to be assembled are related, wholly or in part, and may be derived from a single or many different transcripts. Assembly of the sequences can be performed using such programs as PHRAP (Phils Revised Assembly Program) and the GELVIEW fragment assembly system (GCG), or other methods known in the art.

Alternatively, cDNA sequences are used as "component" sequences that are assembled into "template" or "consensus" sequences as follows. Sequence chromatograms are processed, verified, and quality scores are obtained using PHRED. Raw sequences are edited using an editing pathway known as Block 1 (See, e.g., the LIFESEQ Assembled User Guide, Incyte Genomics, Palo Alto, CA). A series of BLAST comparisons is performed and low-information segments and repetitive elements (e.g., dinucleotide repeats, Alu repeats, etc.) are replaced by "n's", or masked, to prevent spurious matches. Mitochondrial and ribosomal RNA sequences are also removed. The processed sequences are then loaded into a relational database management system (RDMS) which assigns edited sequences to existing templates, if available. When additional sequences are added into the RDMS, a process is initiated which modifies existing templates or creates new templates from works in progress (i.e., nonfinal assembled sequences) containing queued sequences or the sequences themselves. After the new sequences have been assigned to templates, the templates can be merged into bins. If multiple templates exist in one bin, the bin can be split and the templates reannotated.

Once gene bins have been generated based upon sequence alignments, bins are "clone joined" based upon clone information. Clone joining occurs when the 5' sequence of one clone is present in one bin and the 3' sequence from the same clone is present in a different bin, indicating that the two bins should be merged into a single bin. Only bins which share at least two different clones are merged.

5 A resultant template sequence may contain either a partial or a full length open reading frame, or all or part of a genetic regulatory element. This variation is due in part to the fact that the full length cDNAs of many genes are several hundred, and sometimes several thousand, bases in length. With current technology, cDNAs comprising the coding regions of large genes cannot be cloned because of vector limitations, incomplete reverse transcription of the mRNA, or incomplete "second strand"
10 synthesis. Template sequences may be extended to include additional contiguous sequences derived from the parent RNA transcript using a variety of methods known to those of skill in the art. Extension may thus be used to achieve the full length coding sequence of a gene.

Analysis of the cDNA Sequences

15 The cDNA sequences are analyzed using a variety of programs and algorithms which are well known in the art. (See, e.g., Ausubel, 1997, supra, Chapter 7.7; Meyers, R.A. (Ed.) (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853; and Table 7.) These analyses comprise both reading frame determinations, e.g., based on triplet codon periodicity for particular organisms (Fickett, J.W. (1982) *Nucleic Acids Res.* 10:5303-5318); analyses of potential start and stop
20 codons; and homology searches.

Computer programs known to those of skill in the art for performing computer-assisted searches for amino acid and nucleic acid sequence similarity, include, for example, Basic Local Alignment Search Tool (BLAST; Altschul, S.F. (1993) *J. Mol. Evol.* 36:290-300; Altschul, S.F. et al. (1990) *J. Mol. Biol.* 215:403-410). BLAST is especially useful in determining exact matches and
25 comparing two sequence fragments of arbitrary but equal lengths, whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user (Karlin, S. et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:841-845). Using an appropriate search tool (e.g., BLAST or HMM), GenBank, SwissProt, BLOCKS, PFAM and other databases may be searched for sequences containing regions of homology to a query dithp or DITHP of the present invention.

30 Other approaches to the identification, assembly, storage, and display of nucleotide and polypeptide sequences are provided in "Relational Database for Storing Biomolecule Information," U.S.S.N. 08/947,845, filed October 9, 1997; "Project-Based Full-Length Biomolecular Sequence Database," U.S.S.N. 08/811,758, filed March 6, 1997; and "Relational Database and System for

Storing Information Relating to Biomolecular Sequences,” U.S.S.N. 09/034,807, filed March 4, 1998, all of which are incorporated by reference herein in their entirety.

Protein hierarchies can be assigned to the putative encoded polypeptide based on, e.g., motif, BLAST, or biological analysis. Methods for assigning these hierarchies are described, for example, in
5 “Database System Employing Protein Function Hierarchies for Viewing Biomolecular Sequence Data,” U.S.S.N. 08/812,290, filed March 6, 1997, incorporated herein by reference.

Identification of Human Diagnostic and Therapeutic Molecules Encoded by dithp

The identities of the DITHP encoded by the dithp of the present invention were obtained by
10 analysis of the assembled cDNA sequences.

SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215, SEQ ID NO:216, SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, and SEQ ID NO:223, encoded by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10,
15 SEQ ID NO:11, and SEQ ID NO:12, respectively, are, for example, human enzyme molecules.

SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:226, SEQ ID NO:227, and SEQ ID NO:228, encoded by SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, respectively, are, for example, receptor molecules.

SEQ ID NO:229, SEQ ID NO:230, SEQ ID NO:231, SEQ ID NO:232, SEQ ID NO:233, SEQ ID NO:234, SEQ ID NO:235, SEQ ID NO:236, SEQ ID NO:237, SEQ ID NO:238, SEQ ID NO:239, SEQ ID NO:240, and SEQ ID NO:241, encoded by SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, respectively, are, for example, intracellular signaling molecules.

SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:255, SEQ ID NO:256, SEQ ID NO:257, SEQ ID NO:258, SEQ ID NO:259, SEQ ID NO:260, SEQ ID NO:261, SEQ ID NO:262, SEQ ID NO:263, SEQ ID NO:264, SEQ ID NO:265, SEQ ID NO:266, SEQ ID NO:267, SEQ ID NO:268,
25 SEQ ID NO:269, SEQ ID NO:270, SEQ ID NO:271, SEQ ID NO:272, SEQ ID NO:273, SEQ ID NO:274, SEQ ID NO:275, SEQ ID NO:276, SEQ ID NO:277, SEQ ID NO:278, SEQ ID NO:279, SEQ ID NO:280, SEQ ID NO:281, SEQ ID NO:282, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:285, SEQ ID NO:286, SEQ ID NO:287, SEQ ID NO:288, SEQ ID NO:289, SEQ ID NO:290, SEQ ID NO:291, SEQ ID NO:292, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:295, SEQ ID

NO:296, SEQ ID NO:297, SEQ ID NO:298, SEQ ID NO:299, SEQ ID NO:300, SEQ ID NO:301, SEQ ID NO:302, SEQ ID NO:303, SEQ ID NO:304, SEQ ID NO:305, SEQ ID NO:306, SEQ ID NO:307, SEQ ID NO:308, and SEQ ID NO:309, encoded by SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, and SEQ ID NO:98, respectively, are, for example, transcription factor molecules.

SEQ ID NO:310, SEQ ID NO:311, SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:314, SEQ ID NO:315, SEQ ID NO:316, and SEQ ID NO:317, encoded by SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, and SEQ ID NO:106, respectively, are, for example, membrane transport molecules.

SEQ ID NO:318, SEQ ID NO:319, SEQ ID NO:320, SEQ ID NO:321, SEQ ID NO:322, SEQ ID NO:323, SEQ ID NO:324, SEQ ID NO:325, SEQ ID NO:326, and SEQ ID NO:327, encoded by SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, and SEQ ID NO:116, respectively, are, for example, protein modification and maintenance molecules.

SEQ ID NO:328, SEQ ID NO:329, SEQ ID NO:330, SEQ ID NO:331, SEQ ID NO:332, SEQ ID NO:333, SEQ ID NO:334, SEQ ID NO:335, SEQ ID NO:336, SEQ ID NO:337, SEQ ID NO:338, SEQ ID NO:339, SEQ ID NO:340, and SEQ ID NO:341, encoded by SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, and SEQ ID NO:130, respectively, are, for example, nucleic acid synthesis and modification molecules.

SEQ ID NO:342, encoded by SEQ ID NO:131 is, for example, an adhesion molecule.

SEQ ID NO:343, SEQ ID NO:344, SEQ ID NO:345, SEQ ID NO:346, SEQ ID NO:347, SEQ ID NO:348, and SEQ ID NO:349, encoded by SEQ ID NO:132, SEQ ID NO:133, SEQ ID

NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, and SEQ ID NO:138, respectively, are, for example, antigen recognition molecules.

SEQ ID NO:350, SEQ ID NO:351, SEQ ID NO:352, and SEQ ID NO:353, encoded by SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, and SEQ ID NO:142, respectively, are, for example,
5 electron transfer associated molecules.

SEQ ID NO:354, SEQ ID NO:355, SEQ ID NO:356, SEQ ID NO:357, SEQ ID NO:358, and SEQ ID NO:359, encoded by SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, and SEQ ID NO:148, respectively, are, for example, secreted/extracellular matrix molecules.

10 SEQ ID NO:360, SEQ ID NO:361, SEQ ID NO:362, SEQ ID NO:363, SEQ ID NO:364, SEQ ID NO:365, SEQ ID NO:366, SEQ ID NO:367, SEQ ID NO:368, and SEQ ID NO:369, encoded by SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, and SEQ ID NO:158, respectively, are, for example, cytoskeletal molecules.

15 SEQ ID NO:370, SEQ ID NO:371, SEQ ID NO:372, and SEQ ID NO:373, encoded by SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, and SEQ ID NO:162, respectively, are, for example, cell membrane molecules.

SEQ ID NO:374, SEQ ID NO:375, SEQ ID NO:376, SEQ ID NO:377, SEQ ID NO:378, SEQ ID NO:379, SEQ ID NO:380, SEQ ID NO:381, SEQ ID NO:382, SEQ ID NO:383, SEQ ID
20 NO:384, SEQ ID NO:385, SEQ ID NO:386, SEQ ID NO:387, SEQ ID NO:388, SEQ ID NO:389, SEQ ID NO:390, SEQ ID NO:391, and SEQ ID NO:392, encoded by SEQ ID NO:163, SEQ ID NO:164, SEQ ID NO:165, SEQ ID NO:166, SEQ ID NO:167, SEQ ID NO:168, SEQ ID NO:169, SEQ ID NO:170, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173, SEQ ID NO:174, SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, SEQ ID NO:178, SEQ ID NO:179, SEQ ID NO:180,
25 and SEQ ID NO:181, respectively, are, for example, ribosomal molecules.

SEQ ID NO:393, SEQ ID NO:394, SEQ ID NO:395, SEQ ID NO:396, SEQ ID NO:397, SEQ ID NO:398, SEQ ID NO:399, SEQ ID NO:400, SEQ ID NO:401, SEQ ID NO:402, and SEQ ID NO:403, encoded by SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:191,
30 and SEQ ID NO:192, respectively, are, for example, organelle associated molecules.

SEQ ID NO:404, SEQ ID NO:405, SEQ ID NO:406, SEQ ID NO:407, SEQ ID NO:408, SEQ ID NO:409, SEQ ID NO:410, SEQ ID NO:411, SEQ ID NO:412, SEQ ID NO:413, and SEQ ID NO:414, encoded by SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID

NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ ID NO:202, and SEQ ID NO:203, respectively, are, for example, biochemical pathway molecules.

SEQ ID NO:415, SEQ ID NO:416, SEQ ID NO:417, SEQ ID NO:418, SEQ ID NO:419, SEQ ID NO:420, SEQ ID NO:421, and SEQ ID NO:422, encoded by SEQ ID NO:204, SEQ ID
 5 NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, and SEQ ID NO:211, respectively, are, for example, molecules associated with growth and development.

Sequences of Human Diagnostic and Therapeutic Molecules

10 The dithp of the present invention may be used for a variety of diagnostic and therapeutic purposes. For example, a dithp may be used to diagnose a particular condition, disease, or disorder associated with human molecules. Such conditions, diseases, and disorders include, but are not limited to, a cell proliferative disorder, such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal
 15 hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, a cancer of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; an
 20 autoimmune/inflammatory disorder, such as inflammation, actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, bursitis, cholecystitis, cirrhosis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus,
 25 emphysema, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome, episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, myelofibrosis, osteoarthritis, osteoporosis, pancreatitis,
 30 polycythemia vera, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, trauma, and hematopoietic cancer including lymphoma, leukemia, and myeloma; an infection caused by a viral agent classified

as adenovirus, arenavirus, bunyavirus, calicivirus, coronavirus, filovirus, hepadnavirus, herpesvirus, flavivirus, orthomyxovirus, parvovirus, papovavirus, paramyxovirus, picornavirus, poxvirus, reovirus, retrovirus, rhabdovirus, or togavirus; an infection caused by a bacterial agent classified as pneumococcus, staphylococcus, streptococcus, bacillus, corynebacterium, clostridium, meningococcus, gonococcus, listeria, moraxella, kingella, haemophilus, legionella, bordetella, gram-negative enterobacterium including shigella, salmonella, or campylobacter, pseudomonas, vibrio, brucella, francisella, yersinia, bartonella, norcardium, actinomyces, mycobacterium, spirochaetale, rickettsia, chlamydia, or mycoplasma; an infection caused by a fungal agent classified as aspergillus, blastomyces, dermatophytes, cryptococcus, coccidioides, malassezia, histoplasma, or other mycosis-causing fungal agent; and an infection caused by a parasite classified as plasmodium or malaria-causing, parasitic entamoeba, leishmania, trypanosoma, toxoplasma, pneumocystis carinii, intestinal protozoa such as giardia, trichomonas, tissue nematode such as trichinella, intestinal nematode such as ascaris, lymphatic filarial nematode, trematode such as schistosoma, and cestode such as tapeworm; a developmental disorder such as renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation), Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spina bifida, anencephaly, craniorachischisis, congenital glaucoma, cataract, and sensorineural hearing loss; an endocrine disorder such as a disorder of the hypothalamus and/or pituitary resulting from lesions such as a primary brain tumor, adenoma, infarction associated with pregnancy, hypophysectomy, aneurysm, vascular malformation, thrombosis, infection, immunological disorder, and complication due to head trauma; a disorder associated with hypopituitarism including hypogonadism, Sheehan syndrome, diabetes insipidus, Kallman's disease, Hand-Schuller-Christian disease, Letterer-Siwe disease, sarcoidosis, empty sella syndrome, and dwarfism; a disorder associated with hyperpituitarism including acromegaly, gigantism, and syndrome of inappropriate antidiuretic hormone (ADH) secretion (SIADH) often caused by benign adenoma; a disorder associated with hypothyroidism including goiter, myxedema, acute thyroiditis associated with bacterial infection, subacute thyroiditis associated with viral infection, autoimmune thyroiditis (Hashimoto's disease), and cretinism; a disorder associated with hyperthyroidism including thyrotoxicosis and its various forms, Grave's disease, pretibial myxedema, toxic multinodular goiter, thyroid carcinoma, and Plummer's disease; a disorder associated with hyperparathyroidism including Conn disease (chronic hypercalcemia); a pancreatic disorder such as Type I or Type II diabetes mellitus and associated complications; a disorder associated

with the adrenals such as hyperplasia, carcinoma, or adenoma of the adrenal cortex, hypertension associated with alkalosis, amyloidosis, hypokalemia, Cushing's disease, Liddle's syndrome, and Arnold-Healy-Gordon syndrome, pheochromocytoma tumors, and Addison's disease; a disorder associated with gonadal steroid hormones such as: in women, abnormal prolactin production, 5 infertility, endometriosis, perturbation of the menstrual cycle, polycystic ovarian disease, hyperprolactinemia, isolated gonadotropin deficiency, amenorrhea, galactorrhea, hermaphroditism, hirsutism and virilization, breast cancer, and, in post-menopausal women, osteoporosis; and, in men, Leydig cell deficiency, male climacteric phase, and germinal cell aplasia, a hypergonadal disorder associated with Leydig cell tumors, androgen resistance associated with absence of androgen receptors, 10 syndrome of 5 α -reductase, and gynecomastia; a metabolic disorder such as Addison's disease, cerebrotendinous xanthomatosis, congenital adrenal hyperplasia, coumarin resistance, cystic fibrosis, diabetes, fatty hepatocirrhosis, fructose-1,6-diphosphatase deficiency, galactosemia, goiter, glucagonoma, glycogen storage diseases, hereditary fructose intolerance, hyperadrenalism, hypoadrenalism, hyperparathyroidism, hypoparathyroidism, hypercholesterolemia, hyperthyroidism, 15 hypoglycemia, hypothyroidism, hyperlipidemia, hyperlipemia, lipid myopathies, lipodystrophies, lysosomal storage diseases, mannosidosis, neuraminidase deficiency, obesity, pentosuria phenylketonuria, pseudovitamin D-deficiency rickets; disorders of carbohydrate metabolism such as congenital type II dyserythropoietic anemia, diabetes, insulin-dependent diabetes mellitus, non-insulin-dependent diabetes mellitus, fructose-1,6-diphosphatase deficiency, galactosemia, 20 glucagonoma, hereditary fructose intolerance, hypoglycemia, mannosidosis, neuraminidase deficiency, obesity, galactose epimerase deficiency, glycogen storage diseases, lysosomal storage diseases, fructosuria, pentosuria, and inherited abnormalities of pyruvate metabolism; disorders of lipid metabolism such as fatty liver, cholestasis, primary biliary cirrhosis, carnitine deficiency, carnitine palmitoyltransferase deficiency, myoadenylate deaminase deficiency, hypertriglyceridemia, 25 lipid storage disorders such Fabry's disease, Gaucher's disease, Niemann-Pick's disease, metachromatic leukodystrophy, adrenoleukodystrophy, GM₂ gangliosidosis, and ceroid lipofuscinosis, abetalipoproteinemia, Tangier disease, hyperlipoproteinemia, diabetes mellitus, lipodystrophy, lipomatosis, acute panniculitis, disseminated fat necrosis, adiposis dolorosa, lipoid adrenal hyperplasia, minimal change disease, lipomas, atherosclerosis, hypercholesterolemia, 30 hypercholesterolemia with hypertriglyceridemia, primary hypoalphalipoproteinemia, hypothyroidism, renal disease, liver disease, lecithin:cholesterol acyltransferase deficiency, cerebrotendinous xanthomatosis, sitosterolemia, hypocholesterolemia, Tay-Sachs disease, Sandhoff's disease, hyperlipidemia, hyperlipemia, lipid myopathies, and obesity; and disorders of copper metabolism such as Menke's disease, Wilson's disease, and Ehlers-Danlos syndrome type IX; a neurological

disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases,

5 bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease, prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental

10 retardation and other developmental disorder of the central nervous system, cerebral palsy, a neuroskeletal disorder, an autonomic nervous system disorder, a cranial nerve disorder, a spinal cord disease, muscular dystrophy and other neuromuscular disorder, a peripheral nervous system disorder, dermatomyositis and polymyositis, inherited, metabolic, endocrine, and toxic myopathy, myasthenia gravis, periodic paralysis, a mental disorder including mood, anxiety, and schizophrenic disorders,

15 seasonal affective disorder (SAD), akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, and Tourette's disorder; a gastrointestinal disorder including ulcerative colitis, gastric and duodenal ulcers, cystinuria, dibasicaminoaciduria, hypercystinuria, lysinuria, hartnup disease, tryptophan malabsorption, methionine malabsorption, histidinuria, iminoglycinuria, dicarboxylicaminoaciduria, cystinosis, renal

20 glycosuria, hypouricemia, familial hypophosphatemic rickets, congenital chloridorrhea, distal renal tubular acidosis, Menkes' disease, Wilson's disease, lethal diarrhea, juvenile pernicious anemia, folate malabsorption, adrenoleukodystrophy, hereditary myoglobinuria, and Zellweger syndrome; a transport disorder such as akinesia, amyotrophic lateral sclerosis, ataxia telangiectasia, cystic fibrosis, Becker's muscular dystrophy, Bell's palsy, Charcot-Marie Tooth disease, diabetes mellitus, diabetes

25 insipidus, diabetic neuropathy, Duchenne muscular dystrophy, hyperkalemic periodic paralysis, normokalemic periodic paralysis, Parkinson's disease, malignant hyperthermia, multidrug resistance, myasthenia gravis, myotonic dystrophy, catatonia, tardive dyskinesia, dystonias, peripheral neuropathy, cerebral neoplasms, prostate cancer, cardiac disorders associated with transport, e.g., angina, bradyarrhythmia, tachyarrhythmia, hypertension, Long QT syndrome, myocarditis,

30 cardiomyopathy, nemaline myopathy, centronuclear myopathy, lipid myopathy, mitochondrial myopathy, thyrotoxic myopathy, ethanol myopathy, dermatomyositis, inclusion body myositis, infectious myositis, and polymyositis, neurological disorders associated with transport, e.g., Alzheimer's disease, amnesia, bipolar disorder, dementia, depression, epilepsy, Tourette's disorder, paranoid psychoses, and schizophrenia, and other disorders associated with transport, e.g.,

neurofibromatosis, postherpetic neuralgia, trigeminal neuropathy, sarcoidosis, sickle cell anemia, cataracts, infertility, pulmonary artery stenosis, sensorineural autosomal deafness, hyperglycemia, hypoglycemia, Grave's disease, goiter, glucose-galactose malabsorption syndrome, hypercholesterolemia, Cushing's disease, and Addison's disease; and a connective tissue disorder
5 such as osteogenesis imperfecta, Ehlers-Danlos syndrome, chondrodysplasias, Marfan syndrome, Alport syndrome, familial aortic aneurysm, achondroplasia, mucopolysaccharidoses, osteoporosis, osteopetrosis, Paget's disease, rickets, osteomalacia, hyperparathyroidism, renal osteodystrophy, osteonecrosis, osteomyelitis, osteoma, osteoid osteoma, osteoblastoma, osteosarcoma, osteochondroma, chondroma, chondroblastoma, chondromyxoid fibroma, chondrosarcoma, fibrous
10 cortical defect, nonossifying fibroma, fibrous dysplasia, fibrosarcoma, malignant fibrous histiocytoma, Ewing's sarcoma, primitive neuroectodermal tumor, giant cell tumor, osteoarthritis, rheumatoid arthritis, ankylosing spondyloarthritis, Reiter's syndrome, psoriatic arthritis, enteropathic arthritis, infectious arthritis, gout, gouty arthritis, calcium pyrophosphate crystal deposition disease, ganglion, synovial cyst, villonodular synovitis, systemic sclerosis, Dupuytren's contracture, hepatic
15 fibrosis, lupus erythematosus, mixed connective tissue disease, epidermolysis bullosa simplex, bullous congenital ichthyosiform erythroderma (epidermolytic hyperkeratosis), non-epidermolytic and epidermolytic palmoplantar keratoderma, ichthyosis bullosa of Siemens, pachyonychia congenita, and white sponge nevus. The dithp can be used to detect the presence of, or to quantify the amount of, a dithp-related polynucleotide in a sample. This information is then compared to information obtained
20 from appropriate reference samples, and a diagnosis is established. Alternatively, a polynucleotide complementary to a given dithp can inhibit or inactivate a therapeutically relevant gene related to the dithp.

Analysis of dithp Expression Patterns

25 The expression of dithp may be routinely assessed by hybridization-based methods to determine, for example, the tissue-specificity, disease-specificity, or developmental stage-specificity of dithp expression. For example, the level of expression of dithp may be compared among different cell types or tissues, among diseased and normal cell types or tissues, among cell types or tissues at different developmental stages, or among cell types or tissues undergoing various treatments. This type
30 of analysis is useful, for example, to assess the relative levels of dithp expression in fully or partially differentiated cells or tissues, to determine if changes in dithp expression levels are correlated with the development or progression of specific disease states, and to assess the response of a cell or tissue to a specific therapy, for example, in pharmacological or toxicological studies. Methods for the analysis of dithp expression are based on hybridization and amplification technologies and include membrane-

based procedures such as northern blot analysis, high-throughput procedures that utilize, for example, microarrays, and PCR-based procedures.

Hybridization and Genetic Analysis

5 The dithp, their fragments, or complementary sequences, may be used to identify the presence of and/or to determine the degree of similarity between two (or more) nucleic acid sequences. The dithp may be hybridized to naturally occurring or recombinant nucleic acid sequences under appropriately selected temperatures and salt concentrations. Hybridization with a probe based on the nucleic acid sequence of at least one of the dithp allows for the detection of nucleic acid sequences, including
10 genomic sequences, which are identical or related to the dithp of the Sequence Listing. Probes may be selected from non-conserved or unique regions of at least one of the polynucleotides of SEQ ID NO:1-211 and tested for their ability to identify or amplify the target nucleic acid sequence using standard protocols.

Polynucleotide sequences that are capable of hybridizing, in particular, to those shown in SEQ
15 ID NO:1-211 and fragments thereof, can be identified using various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) Hybridization conditions are discussed in "Definitions."

A probe for use in Southern or northern hybridization may be derived from a fragment of a dithp sequence, or its complement, that is up to several hundred nucleotides in length and is either
20 single-stranded or double-stranded. Such probes may be hybridized in solution to biological materials such as plasmids, bacterial, yeast, or human artificial chromosomes, cleared or sectioned tissues, or to artificial substrates containing dithp. Microarrays are particularly suitable for identifying the presence of and detecting the level of expression for multiple genes of interest by examining gene expression correlated with, e.g., various stages of development, treatment with a drug or compound, or disease
25 progression. An array analogous to a dot or slot blot may be used to arrange and link polynucleotides to the surface of a substrate using one or more of the following: mechanical (vacuum), chemical, thermal, or UV bonding procedures. Such an array may contain any number of dithp and may be produced by hand or by using available devices, materials, and machines.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g.,
30 Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

Probes may be labeled by either PCR or enzymatic techniques using a variety of commercially

available reporter molecules. For example, commercial kits are available for radioactive and chemiluminescent labeling (Amersham Pharmacia Biotech) and for alkaline phosphatase labeling (Life Technologies). Alternatively, dithp may be cloned into commercially available vectors for the production of RNA probes. Such probes may be transcribed in the presence of at least one labeled
5 nucleotide (e.g., ^{32}P -ATP, Amersham Pharmacia Biotech).

Additionally the polynucleotides of SEQ ID NO:1-211 or suitable fragments thereof can be used to isolate full length cDNA sequences utilizing hybridization and/or amplification procedures well known in the art, e.g., cDNA library screening, PCR amplification, etc. The molecular cloning of such full length cDNA sequences may employ the method of cDNA library screening with probes using the
10 hybridization, stringency, washing, and probing strategies described above and in Ausubel, supra, Chapters 3, 5, and 6. These procedures may also be employed with genomic libraries to isolate genomic sequences of dithp in order to analyze, e.g., regulatory elements.

Genetic Mapping

15 Gene identification and mapping are important in the investigation and treatment of almost all conditions, diseases, and disorders. Cancer, cardiovascular disease, Alzheimer's disease, arthritis, diabetes, and mental illnesses are of particular interest. Each of these conditions is more complex than the single gene defects of sickle cell anemia or cystic fibrosis, with select groups of genes being predictive of predisposition for a particular condition, disease, or disorder. For example,
20 cardiovascular disease may result from malfunctioning receptor molecules that fail to clear cholesterol from the bloodstream, and diabetes may result when a particular individual's immune system is activated by an infection and attacks the insulin-producing cells of the pancreas. In some studies, Alzheimer's disease has been linked to a gene on chromosome 21; other studies predict a different gene and location. Mapping of disease genes is a complex and reiterative process and generally proceeds
25 from genetic linkage analysis to physical mapping.

As a condition is noted among members of a family, a genetic linkage map traces parts of chromosomes that are inherited in the same pattern as the condition. Statistics link the inheritance of particular conditions to particular regions of chromosomes, as defined by RFLP or other markers. (See, for example, Lander, E. S. and Botstein, D. (1986) Proc. Natl. Acad. Sci. USA 83:7353-7357.)
30 Occasionally, genetic markers and their locations are known from previous studies. More often, however, the markers are simply stretches of DNA that differ among individuals. Examples of genetic linkage maps can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site.

In another embodiment of the invention, dithp sequences may be used to generate hybridization

probes useful in chromosomal mapping of naturally occurring genomic sequences. Either coding or noncoding sequences of dithp may be used, and in some instances, noncoding sequences may be preferable over coding sequences. For example, conservation of a dithp coding sequence among members of a multi-gene family may potentially cause undesired cross hybridization during chromosomal mapping. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Meyers, supra, pp. 965-968.) Correlation between the location of dithp on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The dithp sequences may also be used to detect polymorphisms that are genetically linked to the inheritance of a particular condition, disease, or disorder.

In situ hybridization of chromosomal preparations and genetic mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending existing genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of the corresponding human chromosome is not known. These new marker sequences can be mapped to human chromosomes and may provide valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once a disease or syndrome has been crudely correlated by genetic linkage with a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequences of the subject invention may also be used to detect differences in chromosomal architecture due to translocation, inversion, etc., among normal, carrier, or affected individuals.

Once a disease-associated gene is mapped to a chromosomal region, the gene must be cloned in order to identify mutations or other alterations (e.g., translocations or inversions) that may be correlated with disease. This process requires a physical map of the chromosomal region containing the disease-gene of interest along with associated markers. A physical map is necessary for determining the nucleotide sequence of and order of marker genes on a particular chromosomal region. Physical mapping techniques are well known in the art and require the generation of overlapping sets of cloned

DNA fragments from a particular organelle, chromosome, or genome. These clones are analyzed to reconstruct and catalog their order. Once the position of a marker is determined, the DNA from that region is obtained by consulting the catalog and selecting clones from that region. The gene of interest is located through positional cloning techniques using hybridization or similar methods.

5

Diagnostic Uses

The dithp of the present invention may be used to design probes useful in diagnostic assays. Such assays, well known to those skilled in the art, may be used to detect or confirm conditions, disorders, or diseases associated with abnormal levels of dithp expression. Labeled probes developed from dithp sequences are added to a sample under hybridizing conditions of desired stringency. In some instances, dithp, or fragments or oligonucleotides derived from dithp, may be used as primers in amplification steps prior to hybridization. The amount of hybridization complex formed is quantified and compared with standards for that cell or tissue. If dithp expression varies significantly from the standard, the assay indicates the presence of the condition, disorder, or disease. Qualitative or quantitative diagnostic methods may include northern, dot blot, or other membrane or dip-stick based technologies or multiple-sample format technologies such as PCR, enzyme-linked immunosorbent assay (ELISA)-like, pin, or chip-based assays.

The probes described above may also be used to monitor the progress of conditions, disorders, or diseases associated with abnormal levels of dithp expression, or to evaluate the efficacy of a particular therapeutic treatment. The candidate probe may be identified from the dithp that are specific to a given human tissue and have not been observed in GenBank or other genome databases. Such a probe may be used in animal studies, preclinical tests, clinical trials, or in monitoring the treatment of an individual patient. In a typical process, standard expression is established by methods well known in the art for use as a basis of comparison, samples from patients affected by the disorder or disease are combined with the probe to evaluate any deviation from the standard profile, and a therapeutic agent is administered and effects are monitored to generate a treatment profile. Efficacy is evaluated by determining whether the expression progresses toward or returns to the standard normal pattern. Treatment profiles may be generated over a period of several days or several months. Statistical methods well known to those skilled in the art may be used to determine the significance of such therapeutic agents.

The polynucleotides are also useful for identifying individuals from minute biological samples, for example, by matching the RFLP pattern of a sample's DNA to that of an individual's DNA. The polynucleotides of the present invention can also be used to determine the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR

primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, an individual can be identified through a unique set of DNA sequences. Once a unique ID database is established for an individual, positive identification of that individual can be made from extremely small tissue samples.

5 In a particular aspect, oligonucleotide primers derived from the dithp of the invention may be used to detect single nucleotide polymorphisms (SNPs). SNPs are substitutions, insertions and deletions that are a frequent cause of inherited or acquired genetic disease in humans. Methods of SNP detection include, but are not limited to, single-stranded conformation polymorphism (SSCP) and fluorescent SSCP (fSSCP) methods. In SSCP, oligonucleotide primers derived from dithp are used to
10 amplify DNA using the polymerase chain reaction (PCR). The DNA may be derived, for example, from diseased or normal tissue, biopsy samples, bodily fluids, and the like. SNPs in the DNA cause differences in the secondary and tertiary structures of PCR products in single-stranded form, and these differences are detectable using gel electrophoresis in non-denaturing gels. In fSSCP, the oligonucleotide primers are fluorescently labeled, which allows detection of the amplimers in high-
15 throughput equipment such as DNA sequencing machines. Additionally, sequence database analysis methods, termed in silico SNP (isSNP), are capable of identifying polymorphisms by comparing the sequences of individual overlapping DNA fragments which assemble into a common consensus sequence. These computer-based methods filter out sequence variations due to laboratory preparation of DNA and sequencing errors using statistical models and automated analyses of DNA sequence
20 chromatograms. In the alternative, SNPs may be detected and characterized by mass spectrometry using, for example, the high throughput MASSARRAY system (Sequenom, Inc., San Diego CA).

DNA-based identification techniques are critical in forensic technology. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc., can be amplified using, e.g., PCR, to identify individuals. (See, e.g., Erlich, H. (1992)
25 PCR Technology, Freeman and Co., New York, NY). Similarly, polynucleotides of the present invention can be used as polymorphic markers.

There is also a need for reagents capable of identifying the source of a particular tissue. Appropriate reagents can comprise, for example, DNA probes or primers prepared from the sequences of the present invention that are specific for particular tissues. Panels of such reagents can identify
30 tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

The polynucleotides of the present invention can also be used as molecular weight markers on nucleic acid gels or Southern blots, as diagnostic probes for the presence of a specific mRNA in a particular cell type, in the creation of subtracted cDNA libraries which aid in the discovery of novel

polynucleotides, in selection and synthesis of oligomers for attachment to an array or other support, and as an antigen to elicit an immune response.

Disease Model Systems Using dithp

5 The dithp of the invention or their mammalian homologs may be “knocked out” in an animal model system using homologous recombination in embryonic stem (ES) cells. Such techniques are well known in the art and are useful for the generation of animal models of human disease. (See, e.g., U.S. Patent Number 5,175,383 and U.S. Patent Number 5,767,337.) For example, mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and grown in culture. The ES
10 cells are transformed with a vector containing the gene of interest disrupted by a marker gene, e.g., the neomycin phosphotransferase gene (neo; Capecchi, M.R. (1989) Science 244:1288-1292). The vector integrates into the corresponding region of the host genome by homologous recombination. Alternatively, homologous recombination takes place using the Cre-loxP system to knockout a gene of interest in a tissue- or developmental stage-specific manner (Marth, J.D. (1996) Clin. Invest. 97:1999-
15 2002; Wagner, K.U. et al. (1997) Nucleic Acids Res. 25:4323-4330). Transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred to pseudopregnant dams, and the resulting chimeric progeny are genotyped and bred to produce heterozygous or homozygous strains. Transgenic animals thus generated may be tested with potential therapeutic or toxic agents.

20 The dithp of the invention may also be manipulated in vitro in ES cells derived from human blastocysts. Human ES cells have the potential to differentiate into at least eight separate cell lineages including endoderm, mesoderm, and ectodermal cell types. These cell lineages differentiate into, for example, neural cells, hematopoietic lineages, and cardiomyocytes (Thomson, J.A. et al. (1998) Science 282:1145-1147).

25 The dithp of the invention can also be used to create “knockin” humanized animals (pigs) or transgenic animals (mice or rats) to model human disease. With knockin technology, a region of dithp is injected into animal ES cells, and the injected sequence integrates into the animal cell genome. Transformed cells are injected into blastulae, and the blastulae are implanted as described above. Transgenic progeny or inbred lines are studied and treated with potential pharmaceutical agents to
30 obtain information on treatment of a human disease. Alternatively, a mammal inbred to overexpress dithp, resulting, e.g., in the secretion of DITHP in its milk, may also serve as a convenient source of that protein (Janne, J. et al. (1998) Biotechnol. Annu. Rev. 4:55-74).

Screening Assays

DITHP encoded by polynucleotides of the present invention may be used to screen for molecules that bind to or are bound by the encoded polypeptides. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the bound molecule. Examples of such molecules include antibodies, oligonucleotides, 5 proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a ligand or fragment thereof, a natural substrate, or a structural or functional mimetic. (See, Coligan et al., (1991) Current Protocols in Immunology 1(2): Chapter 5.) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or to at least a fragment of the receptor, 10 e.g., the active site. In either case, the molecule can be rationally designed using known techniques. Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing the polypeptide or cell membrane fractions which contain the expressed polypeptide are then contacted with a test compound and binding, 15 stimulation, or inhibition of activity of either the polypeptide or the molecule is analyzed.

An assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a fluorophore, radioisotope, enzyme conjugate, or other detectable label. Alternatively, the assay may assess binding in the presence of a labeled competitor.

Additionally, the assay can be carried out using cell-free preparations, polypeptide/molecule 20 affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay using, e.g., a monoclonal or polyclonal antibody, can measure 25 polypeptide level in a sample. The antibody can measure polypeptide level by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

All of the above assays can be used in a diagnostic or prognostic context. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the 30 assays can discover agents which may inhibit or enhance the production of the polypeptide from suitably manipulated cells or tissues.

Transcript Imaging and Toxicological Testing

Another embodiment relates to the use of dithp to develop a transcript image of a tissue or cell

type. A transcript image represents the global pattern of gene expression by a particular tissue or cell type. Global gene expression patterns are analyzed by quantifying the number of expressed genes and their relative abundance under given conditions and at a given time. (See Seilhamer et al., "Comparative Gene Transcript Analysis," U.S. Patent Number 5,840,484, expressly incorporated by reference herein.) Thus a transcript image may be generated by hybridizing the polynucleotides of the present invention or their complements to the totality of transcripts or reverse transcripts of a particular tissue or cell type. In one embodiment, the hybridization takes place in high-throughput format, wherein the polynucleotides of the present invention or their complements comprise a subset of a plurality of elements on a microarray. The resultant transcript image would provide a profile of gene activity pertaining to human molecules for diagnostics and therapeutics.

Transcript images which profile dithp expression may be generated using transcripts isolated from tissues, cell lines, biopsies, or other biological samples. The transcript image may thus reflect dithp expression in vivo, as in the case of a tissue or biopsy sample, or in vitro, as in the case of a cell line.

Transcript images which profile dithp expression may also be used in conjunction with in vitro model systems and preclinical evaluation of pharmaceuticals, as well as toxicological testing of industrial and naturally-occurring environmental compounds. All compounds induce characteristic gene expression patterns, frequently termed molecular fingerprints or toxicant signatures, which are indicative of mechanisms of action and toxicity (Nuwaysir, E. F. et al. (1999) Mol. Carcinog. 24:153-159; Steiner, S. and Anderson, N.L. (2000) Toxicol. Lett. 112-113:467-71, expressly incorporated by reference herein). If a test compound has a signature similar to that of a compound with known toxicity, it is likely to share those toxic properties. These fingerprints or signatures are most useful and refined when they contain expression information from a large number of genes and gene families. Ideally, a genome-wide measurement of expression provides the highest quality signature. Even genes whose expression is not altered by any tested compounds are important as well, as the levels of expression of these genes are used to normalize the rest of the expression data. The normalization procedure is useful for comparison of expression data after treatment with different compounds. While the assignment of gene function to elements of a toxicant signature aids in interpretation of toxicity mechanisms, knowledge of gene function is not necessary for the statistical matching of signatures which leads to prediction of toxicity. (See, for example, Press Release 00-02 from the National Institute of Environmental Health Sciences, released February 29, 2000, available at <http://www.niehs.nih.gov/oc/news/toxchip.htm>.) Therefore, it is important and desirable in toxicological screening using toxicant signatures to include all expressed gene sequences.

In one embodiment, the toxicity of a test compound is assessed by treating a biological sample

containing nucleic acids with the test compound. Nucleic acids that are expressed in the treated biological sample are hybridized with one or more probes specific to the polynucleotides of the present invention, so that transcript levels corresponding to the polynucleotides of the present invention may be quantified. The transcript levels in the treated biological sample are compared with levels in an untreated biological sample. Differences in the transcript levels between the two samples are indicative of a toxic response caused by the test compound in the treated sample.

Another particular embodiment relates to the use of DITHP encoded by polynucleotides of the present invention to analyze the proteome of a tissue or cell type. The term proteome refers to the global pattern of protein expression in a particular tissue or cell type. Each protein component of a proteome can be subjected individually to further analysis. Proteome expression patterns, or profiles, are analyzed by quantifying the number of expressed proteins and their relative abundance under given conditions and at a given time. A profile of a cell's proteome may thus be generated by separating and analyzing the polypeptides of a particular tissue or cell type. In one embodiment, the separation is achieved using two-dimensional gel electrophoresis, in which proteins from a sample are separated by isoelectric focusing in the first dimension, and then according to molecular weight by sodium dodecyl sulfate slab gel electrophoresis in the second dimension (Steiner and Anderson, *supra*). The proteins are visualized in the gel as discrete and uniquely positioned spots, typically by staining the gel with an agent such as Coomassie Blue or silver or fluorescent stains. The optical density of each protein spot is generally proportional to the level of the protein in the sample. The optical densities of equivalently positioned protein spots from different samples, for example, from biological samples either treated or untreated with a test compound or therapeutic agent, are compared to identify any changes in protein spot density related to the treatment. The proteins in the spots are partially sequenced using, for example, standard methods employing chemical or enzymatic cleavage followed by mass spectrometry. The identity of the protein in a spot may be determined by comparing its partial sequence, preferably of at least 5 contiguous amino acid residues, to the polypeptide sequences of the present invention. In some cases, further sequence data may be obtained for definitive protein identification.

A proteomic profile may also be generated using antibodies specific for DITHP to quantify the levels of DITHP expression. In one embodiment, the antibodies are used as elements on a microarray, and protein expression levels are quantified by exposing the microarray to the sample and detecting the levels of protein bound to each array element (Lueking, A. et al. (1999) *Anal. Biochem.* 270:103-111; Mendoze, L.G. et al. (1999) *Biotechniques* 27:778-88). Detection may be performed by a variety of methods known in the art, for example, by reacting the proteins in the sample with a thiol- or amino-reactive fluorescent compound and detecting the amount of fluorescence bound at each array element.

Toxicant signatures at the proteome level are also useful for toxicological screening, and should

be analyzed in parallel with toxicant signatures at the transcript level. There is a poor correlation between transcript and protein abundances for some proteins in some tissues (Anderson, N.L. and Seilhamer, J. (1997) Electrophoresis 18:533-537), so proteome toxicant signatures may be useful in the analysis of compounds which do not significantly affect the transcript image, but which alter the proteomic profile. In addition, the analysis of transcripts in body fluids is difficult, due to rapid degradation of mRNA, so proteomic profiling may be more reliable and informative in such cases.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins that are expressed in the treated biological sample are separated so that the amount of each protein can be quantified. The amount of each protein is compared to the amount of the corresponding protein in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample. Individual proteins are identified by sequencing the amino acid residues of the individual proteins and comparing these partial sequences to the DITHP encoded by polynucleotides of the present invention.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins from the biological sample are incubated with antibodies specific to the DITHP encoded by polynucleotides of the present invention. The amount of protein recognized by the antibodies is quantified. The amount of protein in the treated biological sample is compared with the amount in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample.

Transcript images may be used to profile dithp expression in distinct tissue types. This process can be used to determine human molecule activity in a particular tissue type relative to this activity in a different tissue type. Transcript images may be used to generate a profile of dithp expression characteristic of diseased tissue. Transcript images of tissues before and after treatment may be used for diagnostic purposes, to monitor the progression of disease, and to monitor the efficacy of drug treatments for diseases which affect the activity of human molecules.

Transcript images of cell lines can be used to assess human molecule activity and/or to identify cell lines that lack or misregulate this activity. Such cell lines may then be treated with pharmaceutical agents, and a transcript image following treatment may indicate the efficacy of these agents in restoring desired levels of this activity. A similar approach may be used to assess the toxicity of pharmaceutical agents as reflected by undesirable changes in human molecule activity. Candidate pharmaceutical agents may be evaluated by comparing their associated transcript images with those of pharmaceutical agents of known effectiveness.

Antisense Molecules

The polynucleotides of the present invention are useful in antisense technology. Antisense technology or therapy relies on the modulation of expression of a target protein through the specific binding of an antisense sequence to a target sequence encoding the target protein or directing its expression. (See, e.g., Agrawal, S., ed. (1996) Antisense Therapeutics, Humana Press Inc., Totawa NJ; Alama, A. et al. (1997) *Pharmacol. Res.* 36(3):171-178; Crooke, S.T. (1997) *Adv. Pharmacol.* 40:1-49; Sharma, H.W. and R. Narayanan (1995) *Bioessays* 17(12):1055-1063; and Lavrosky, Y. et al. (1997) *Biochem. Mol. Med.* 62(1):11-22.) An antisense sequence is a polynucleotide sequence capable of specifically hybridizing to at least a portion of the target sequence. Antisense sequences bind to cellular mRNA and/or genomic DNA, affecting translation and/or transcription. Antisense sequences can be DNA, RNA, or nucleic acid mimics and analogs. (See, e.g., Rossi, J.J. et al. (1991) *Antisense Res. Dev.* 1(3):285-288; Lee, R. et al. (1998) *Biochemistry* 37(3):900-1010; Pardridge, W.M. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92(12):5592-5596; and Nielsen, P. E. and Haaima, G. (1997) *Chem. Soc. Rev.* 96:73-78.) Typically, the binding which results in modulation of expression occurs through hybridization or binding of complementary base pairs. Antisense sequences can also bind to DNA duplexes through specific interactions in the major groove of the double helix.

The polynucleotides of the present invention and fragments thereof can be used as antisense sequences to modify the expression of the polypeptide encoded by dithp. The antisense sequences can be produced ex vivo, such as by using any of the ABI nucleic acid synthesizer series (Applied Biosystems) or other automated systems known in the art. Antisense sequences can also be produced biologically, such as by transforming an appropriate host cell with an expression vector containing the sequence of interest. (See, e.g., Agrawal, supra.)

In therapeutic use, any gene delivery system suitable for introduction of the antisense sequences into appropriate target cells can be used. Antisense sequences can be delivered intracellularly in the form of an expression plasmid which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein. (See, e.g., Slater, J.E., et al. (1998) *J. Allergy Clin. Immunol.* 102(3):469-475; and Scanlon, K.J., et al. (1995) 9(13):1288-1296.) Antisense sequences can also be introduced intracellularly through the use of viral vectors, such as retrovirus and adeno-associated virus vectors. (See, e.g., Miller, A.D. (1990) *Blood* 76:271; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY; Uckert, W. and W. Walther (1994) *Pharmacol. Ther.* 63(3):323-347.) Other gene delivery mechanisms include liposome-derived systems, artificial viral envelopes, and other systems known in the art. (See, e.g., Rossi, J.J. (1995) *Br. Med. Bull.* 51(1):217-225; Boado, R.J. et al. (1998) *J. Pharm. Sci.* 87(11):1308-1315; and Morris, M.C. et al. (1997) *Nucleic Acids Res.* 25(14):2730-2736.)

Expression

In order to express a biologically active DITHP, the nucleotide sequences encoding DITHP or fragments thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding DITHP and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, supra, Chapters 4, 8, 16, and 17; and Ausubel, supra, Chapters 9, 10, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding DITHP. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal (mammalian) cell systems. (See, e.g., Sambrook, supra; Ausubel, 1995, supra, Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; Scorer, C.A. et al. (1994) Bio/Technology 12:181-184; Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945; Takamatsu, N. (1987) EMBO J. 6:307-311; Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105; The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196; Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659; and Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.) Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. (See, e.g., Di Nicola, M. et al. (1998) Cancer Gen. Ther. 5(6):350-356; Yu, M. et al., (1993) Proc. Natl. Acad. Sci. USA 90(13):6340-6344; Buller, R.M. et al. (1985) Nature 317(6040):813-815; McGregor, D.P. et al. (1994) Mol. Immunol. 31(3):219-226; and Verma, I.M. and N. Somia (1997) Nature 389:239-242.) The invention is not limited by the host cell employed.

For long term production of recombinant proteins in mammalian systems, stable expression of DITHP in cell lines is preferred. For example, sequences encoding DITHP can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Any number of

selection systems may be used to recover transformed cell lines. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.; Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14; Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051; Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Therapeutic Uses of dithp

The dithp of the invention may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R.M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassemias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R.G. (1995) Science 270:404-410; Verma, I.M. and Somia, N. (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA. 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in dithp expression or regulation causes disease, the expression of dithp from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

In a further embodiment of the invention, diseases or disorders caused by deficiencies in dithp are treated by constructing mammalian expression vectors comprising dithp and introducing these vectors by mechanical means into dithp-deficient cells. Mechanical transfer technologies for use with cells in vivo or ex vitro include (i) direct DNA microinjection into individual cells, (ii) ballistic gold particle delivery, (iii) liposome-mediated transfection, (iv) receptor-mediated gene transfer, and (v) the use of DNA transposons (Morgan, R.A. and Anderson, W.F. (1993) Annu. Rev. Biochem. 62:191-217; Ivics, Z. (1997) Cell 91:501-510; Boulay, J-L. and Récipon, H. (1998) Curr. Opin. Biotechnol. 9:445-450).

Expression vectors that may be effective for the expression of dithp include, but are not limited

to, the PCDNA 3.1, EPITAG, PRCCMV2, PREP, PVAX vectors (Invitrogen, Carlsbad CA), PCMV-SCRIPT, PCMV-TAG, PEGSH/PERV (Stratagene, La Jolla CA), and PTET-OFF, PTET-ON, PTRE2, PTRE2-LUC, PTK-HYG (Clontech, Palo Alto CA). The dithp of the invention may be expressed using (i) a constitutively active promoter, (e.g., from cytomegalovirus (CMV), Rous sarcoma virus (RSV), SV40 virus, thymidine kinase (TK), or β -actin genes), (ii) an inducible promoter (e.g., the tetracycline-regulated promoter (Gossen, M. and Bujard, H. (1992) Proc. Natl. Acad. Sci. U.S.A. 89:5547-5551; Gossen, M. et al., (1995) Science 268:1766-1769; Rossi, F.M.V. and Blau, H.M. (1998) Curr. Opin. Biotechnol. 9:451-456), commercially available in the T-REX plasmid (Invitrogen); the ecdysone-inducible promoter (available in the plasmids PVGRXR and PIND; 5
10
Invitrogen); the FK506/rapamycin inducible promoter; or the RU486/mifepristone inducible promoter (Rossi, F.M.V. and Blau, H.M. supra), or (iii) a tissue-specific promoter or the native promoter of the endogenous gene encoding DITHP from a normal individual.

Commercially available liposome transformation kits (e.g., the PERFECT LIPID TRANSFECTION KIT, available from Invitrogen) allow one with ordinary skill in the art to deliver 15
polynucleotides to target cells in culture and require minimal effort to optimize experimental parameters. In the alternative, transformation is performed using the calcium phosphate method (Graham, F.L. and Eb, A.J. (1973) Virology 52:456-467), or by electroporation (Néumann, E. et al. (1982) EMBO J. 1:841-845). The introduction of DNA to primary cells requires modification of these standardized mammalian transfection protocols.

20 In another embodiment of the invention, diseases or disorders caused by genetic defects with respect to dithp expression are treated by constructing a retrovirus vector consisting of (i) dithp under the control of an independent promoter or the retrovirus long terminal repeat (LTR) promoter, (ii) appropriate RNA packaging signals, and (iii) a Rev-responsive element (RRE) along with additional retrovirus *cis*-acting RNA sequences and coding sequences required for efficient vector propagation. 25
Retrovirus vectors (e.g., PFB and PFBNEO) are commercially available (Stratagene) and are based on published data (Riviere, I. et al. (1995) Proc. Natl. Acad. Sci. U.S.A. 92:6733-6737), incorporated by reference herein. The vector is propagated in an appropriate vector producing cell line (VPCL) that expresses an envelope gene with a tropism for receptors on the target cells or a promiscuous envelope protein such as VSVg (Armentano, D. et al. (1987) J. Virol. 61:1647-1650; Bender, M.A. et al. (1987) 30
J. Virol. 61:1639-1646; Adam, M.A. and Miller, A.D. (1988) J. Virol. 62:3802-3806; Dull, T. et al. (1998) J. Virol. 72:8463-8471; Zufferey, R. et al. (1998) J. Virol. 72:9873-9880). U.S. Patent Number 5,910,434 to Rigg ("Method for obtaining retrovirus packaging cell lines producing high transducing efficiency retroviral supernatant") discloses a method for obtaining retrovirus packaging cell lines and is hereby incorporated by reference. Propagation of retrovirus vectors, transduction of a population of

cells (e.g., CD4⁺ T-cells), and the return of transduced cells to a patient are procedures well known to persons skilled in the art of gene therapy and have been well documented (Ranga, U. et al. (1997) J. Virol. 71:7020-7029; Bauer, G. et al. (1997) Blood 89:2259-2267; Bonyhadi, M.L. (1997) J. Virol. 71:4707-4716; Ranga, U. et al. (1998) Proc. Natl. Acad. Sci. U.S.A. 95:1201-1206; Su, L. (1997) Blood 89:2283-2290).

In the alternative, an adenovirus-based gene therapy delivery system is used to deliver dithp to cells which have one or more genetic abnormalities with respect to the expression of dithp. The construction and packaging of adenovirus-based vectors are well known to those with ordinary skill in the art. Replication defective adenovirus vectors have proven to be versatile for importing genes encoding immunoregulatory proteins into intact islets in the pancreas (Csete, M.E. et al. (1995) Transplantation 27:263-268). Potentially useful adenoviral vectors are described in U.S. Patent Number 5,707,618 to Armentano ("Adenovirus vectors for gene therapy"), hereby incorporated by reference. For adenoviral vectors, see also Antinozzi, P.A. et al. (1999) Annu. Rev. Nutr. 19:511-544 and Verma, I.M. and Somia, N. (1997) Nature 18:389:239-242, both incorporated by reference herein.

In another alternative, a herpes-based, gene therapy delivery system is used to deliver dithp to target cells which have one or more genetic abnormalities with respect to the expression of dithp. The use of herpes simplex virus (HSV)-based vectors may be especially valuable for introducing dithp to cells of the central nervous system, for which HSV has a tropism. The construction and packaging of herpes-based vectors are well known to those with ordinary skill in the art. A replication-competent herpes simplex virus (HSV) type 1-based vector has been used to deliver a reporter gene to the eyes of primates (Liu, X. et al. (1999) Exp. Eye Res. 169:385-395). The construction of a HSV-1 virus vector has also been disclosed in detail in U.S. Patent Number 5,804,413 to DeLuca ("Herpes simplex virus strains for gene transfer"), which is hereby incorporated by reference. U.S. Patent Number 5,804,413 teaches the use of recombinant HSV d92 which consists of a genome containing at least one exogenous gene to be transferred to a cell under the control of the appropriate promoter for purposes including human gene therapy. Also taught by this patent are the construction and use of recombinant HSV strains deleted for ICP4, ICP27 and ICP22. For HSV vectors, see also Goins, W. F. et al. 1999 J. Virol. 73:519-532 and Xu, H. et al., (1994) Dev. Biol. 163:152-161, hereby incorporated by reference. The manipulation of cloned herpesvirus sequences, the generation of recombinant virus following the transfection of multiple plasmids containing different segments of the large herpesvirus genomes, the growth and propagation of herpesvirus, and the infection of cells with herpesvirus are techniques well known to those of ordinary skill in the art.

In another alternative, an alphavirus (positive, single-stranded RNA virus) vector is used to deliver dithp to target cells. The biology of the prototypic alphavirus, Semliki Forest Virus (SFV), has

been studied extensively and gene transfer vectors have been based on the SFV genome (Garoff, H. and Li, K.-J. (1998) *Curr. Opin. Biotech.* 9:464-469). During alphavirus RNA replication, a subgenomic RNA is generated that normally encodes the viral capsid proteins. This subgenomic RNA replicates to higher levels than the full-length genomic RNA, resulting in the overproduction of capsid proteins relative to the viral proteins with enzymatic activity (e.g., protease and polymerase). Similarly, inserting dithp into the alphavirus genome in place of the capsid-coding region results in the production of a large number of dithp RNAs and the synthesis of high levels of DITHP in vector transduced cells. While alphavirus infection is typically associated with cell lysis within a few days, the ability to establish a persistent infection in hamster normal kidney cells (BHK-21) with a variant of Sindbis virus (SIN) indicates that the lytic replication of alphaviruses can be altered to suit the needs of the gene therapy application (Dryga, S.A. et al. (1997) *Virology* 228:74-83). The wide host range of alphaviruses will allow the introduction of dithp into a variety of cell types. The specific transduction of a subset of cells in a population may require the sorting of cells prior to transduction. The methods of manipulating infectious cDNA clones of alphaviruses, performing alphavirus cDNA and RNA transfections, and performing alphavirus infections, are well known to those with ordinary skill in the art.

Antibodies

Anti-DITHP antibodies may be used to analyze protein expression levels. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, and Fab fragments. For descriptions of and protocols of antibody technologies; see, e.g., Pound J.D. (1998) Immunochemical Protocols, Humana Press, Totowa, NJ.

The amino acid sequence encoded by the dithp of the Sequence Listing may be analyzed by appropriate software (e.g., LASERGENE NAVIGATOR software, DNASTAR) to determine regions of high immunogenicity. The optimal sequences for immunization are selected from the C-terminus, the N-terminus, and those intervening, hydrophilic regions of the polypeptide which are likely to be exposed to the external environment when the polypeptide is in its natural conformation. Analysis used to select appropriate epitopes is also described by Ausubel (1997, supra, Chapter 11.7). Peptides used for antibody induction do not need to have biological activity; however, they must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at least five amino acids, preferably at least 10 amino acids, and most preferably at least 15 amino acids. A peptide which mimics an antigenic fragment of the natural polypeptide may be fused with another protein such as keyhole limpet hemocyanin (KLH; Sigma, St. Louis MO) for antibody production. A peptide encompassing an antigenic region may be expressed from a dithp, synthesized as described above, or

purified from human cells.

Procedures well known in the art may be used for the production of antibodies. Various hosts including mice, goats, and rabbits, may be immunized by injection with a peptide. Depending on the host species, various adjuvants may be used to increase immunological response.

5 In one procedure, peptides about 15 residues in length may be synthesized using an ABI 431A peptide synthesizer (Applied Biosystems) using fmoc-chemistry and coupled to KLH (Sigma) by reaction with M-maleimidobenzoyl-N-hydroxysuccinimide ester (Ausubel, 1995, supra). Rabbits are immunized with the peptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity by binding the peptide to plastic, blocking with 1% bovine serum albumin
10 (BSA), reacting with rabbit antisera, washing, and reacting with radioiodinated goat anti-rabbit IgG. Antisera with antipeptide activity are tested for anti-DITHP activity using protocols well known in the art, including ELISA, radioimmunoassay (RIA), and immunoblotting.

In another procedure, isolated and purified peptide may be used to immunize mice (about 100 µg of peptide) or rabbits (about 1 mg of peptide). Subsequently, the peptide is radioiodinated and used
15 to screen the immunized animals' B-lymphocytes for production of antipeptide antibodies. Positive cells are then used to produce hybridomas using standard techniques. About 20 mg of peptide is sufficient for labeling and screening several thousand clones. Hybridomas of interest are detected by screening with radioiodinated peptide to identify those fusions producing peptide-specific monoclonal antibody. In a typical protocol, wells of a multi-well plate (FAST, Becton-Dickinson, Palo Alto, CA)
20 are coated with affinity-purified, specific rabbit-anti-mouse (or suitable anti-species IgG) antibodies at 10 mg/ml. The coated wells are blocked with 1% BSA and washed and exposed to supernatants from hybridomas. After incubation, the wells are exposed to radiolabeled peptide at 1 mg/ml.

Clones producing antibodies bind a quantity of labeled peptide that is detectable above background. Such clones are expanded and subjected to 2 cycles of cloning. Cloned hybridomas are
25 injected into pristane-treated mice to produce ascites, and monoclonal antibody is purified from the ascitic fluid by affinity chromatography on protein A (Amersham Pharmacia Biotech). Several procedures for the production of monoclonal antibodies, including in vitro production, are described in Pound (supra). Monoclonal antibodies with antipeptide activity are tested for anti-DITHP activity using protocols well known in the art, including ELISA, RIA, and immunoblotting.

30 Antibody fragments containing specific binding sites for an epitope may also be generated. For example, such fragments include, but are not limited to, the F(ab')₂ fragments produced by pepsin digestion of the antibody molecule, and the Fab fragments generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, construction of Fab expression libraries in filamentous bacteriophage allows rapid and easy identification of monoclonal fragments with desired specificity

(Pound, supra, Chaps. 45-47). Antibodies generated against polypeptide encoded by dithp can be used to purify and characterize full-length DITHP protein and its activity, binding partners, etc.

Assays Using Antibodies

5 Anti-DITHP antibodies may be used in assays to quantify the amount of DITHP found in a particular human cell. Such assays include methods utilizing the antibody and a label to detect expression level under normal or disease conditions. The peptides and antibodies of the invention may be used with or without modification or labeled by joining them, either covalently or noncovalently, with a reporter molecule.

10 Protocols for detecting and measuring protein expression using either polyclonal or monoclonal antibodies are well known in the art. Examples include ELISA, RIA, and fluorescent activated cell sorting (FACS). Such immunoassays typically involve the formation of complexes between the DITHP and its specific antibody and the measurement of such complexes. These and other assays are described in Pound (supra).

15 Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

 The disclosures of all patents, applications, and publications mentioned above and below,
20 including U.S. Ser. No. 60/184,777, U.S. Ser. No. 60/184,797, U.S. Ser. No. 60/184,698, U.S. Ser. No. 60/184,770, U.S. Ser. No. 60/184,774, U.S. Ser. No. 60/184,693, U.S. Ser. No. 60/184,771, U.S. Ser. No. 60/184,813, U.S. Ser. No. 60/184,773, U.S. Ser. No. 60/184,776, U.S. Ser. No. 60/184,769, U.S. Ser. No. 60/184,768, U.S. Ser. No. 60/184,837, U.S. Ser. No. 60/184,697, U.S. Ser. No. 60/184,841, U.S. Ser. No. 60/184,772, U.S. Ser. No. 60/185,213, U.S. Ser. No. 60/185,216, U.S. Ser.
25 No. 60/204,863, U.S. Ser. No. 60/205,221, U.S. Ser. No. 60/204,815, U.S. Ser. No. 60/203,785, U.S. Ser. No. 60/204,821, U.S. Ser. No. 60/204,908, U.S. Ser. No. 60/204,226, U.S. Ser. No. 60/204,525, U.S. Ser. No. 60/205,285, U.S. Ser. No. 60/205,232, U.S. Ser. No. 60/205,323, U.S. Ser. No. 60/205,287, U.S. Ser. No. 60/205,324, and U.S. Ser. No. 60/205,286, are hereby expressly incorporated by reference.

30

EXAMPLES

I. Construction of cDNA Libraries

RNA was purchased from CLONTECH Laboratories, Inc. (Palo Alto CA) or isolated from various tissues. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others

were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated with either isopropanol or sodium acetate and ethanol, or by other routine methods.

5 Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In most cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega Corporation (Promega), Madison WI), OLIGOTEX latex particles (QIAGEN, Inc. (QIAGEN), Valencia CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other
10 RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Inc., Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene Cloning Systems, Inc. (Stratagene), La Jolla CA) or SUPERSCRIP^T plasmid system (Life Technologies), using the recommended procedures or similar methods known in
15 the art. (See, e.g., Ausubel, 1997, supra, Chapters 5.1 through 6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or
20 preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSPORT1 plasmid (Life Technologies), PCDNA2.1 plasmid (Invitrogen, Carlsbad CA), PBK-CMV plasmid (Stratagene), or pINCY (Incyte Genomics, Palo Alto CA), or derivatives thereof. Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from
25 Stratagene or DH5 α , DH10B, or ElectroMAX DH10B from Life Technologies.

II. Isolation of cDNA Clones

Plasmids were recovered from host cells by in vivo excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: the Magic or
30 WIZARD Minipreps DNA purification system (Promega); the AGTC Miniprep purification kit (Edge BioSystems, Gaithersburg MD); and the QIAWELL 8, QIAWELL 8 Plus, and QIAWELL 8 Ultra plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit (QIAGEN). Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format. (Rao, V.B. (1994) Anal. Biochem. 216:1-14.) Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using
5 PICOGREEN dye (Molecular Probes, Inc. (Molecular Probes), Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

III. Sequencing and Analysis

cDNA sequencing reactions were processed using standard methods or high-throughput
10 instrumentation such as the ABI CATALYST 800 thermal cycler (Applied Biosystems) or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific Corp., Sunnyvale CA) or the MICROLAB 2200 liquid transfer system (Hamilton). cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit
15 (Applied Biosystems). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (Applied Biosystems) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods
20 (reviewed in Ausubel, 1997, supra, Chapter 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example VIII.

IV. Assembly and Analysis of Sequences

Component sequences from chromatograms were subject to PHRED analysis and assigned a
25 quality score. The sequences having at least a required quality score were subject to various pre-processing editing pathways to eliminate, e.g., low quality 3' ends, vector and linker sequences, polyA tails, Alu repeats, mitochondrial and ribosomal sequences, bacterial contamination sequences, and sequences smaller than 50 base pairs. In particular, low-information sequences and repetitive elements (e.g., dinucleotide repeats, Alu repeats, etc.) were replaced by "n's", or masked, to prevent spurious
30 matches.

Processed sequences were then subject to assembly procedures in which the sequences were assigned to gene bins (bins). Each sequence could only belong to one bin. Sequences in each gene bin were assembled to produce consensus sequences (templates). Subsequent new sequences were added to existing bins using BLASTn (v.1.4 WashU) and CROSSMATCH. Candidate pairs were identified as

all BLAST hits having a quality score greater than or equal to 150. Alignments of at least 82% local identity were accepted into the bin. The component sequences from each bin were assembled using a version of PHRAP. Bins with several overlapping component sequences were assembled using DEEP PHRAP. The orientation (sense or antisense) of each assembled template was determined based on the number and orientation of its component sequences. Template sequences as disclosed in the sequence listing correspond to sense strand sequences (the "forward" reading frames), to the best determination. The complementary (antisense) strands are inherently disclosed herein. The component sequences which were used to assemble each template consensus sequence are listed in Table 4, along with their positions along the template nucleotide sequences.

Bins were compared against each other and those having local similarity of at least 82% were combined and reassembled. Reassembled bins having templates of insufficient overlap (less than 95% local identity) were re-split. Assembled templates were also subject to analysis by STITCHER/EXON MAPPER algorithms which analyze the probabilities of the presence of splice variants, alternatively spliced exons, splice junctions, differential expression of alternative spliced genes across tissue types or disease states, etc. These resulting bins were subject to several rounds of the above assembly procedures.

Once gene bins were generated based upon sequence alignments, bins were clone joined based upon clone information. If the 5' sequence of one clone was present in one bin and the 3' sequence from the same clone was present in a different bin, it was likely that the two bins actually belonged together in a single bin. The resulting combined bins underwent assembly procedures to regenerate the consensus sequences.

The final assembled templates were subsequently annotated using the following procedure. Template sequences were analyzed using BLASTn (v2.0, NCBI) versus gbpri (GenBank version 120). "Hits" were defined as an exact match having from 95% local identity over 200 base pairs through 100% local identity over 100 base pairs, or a homolog match having an E-value, i.e. a probability score, of $\leq 1 \times 10^{-8}$. The hits were subject to frameshift FASTx versus GENPEPT (GenBank version 120). (See Table 7). In this analysis, a homolog match was defined as having an E-value of $\leq 1 \times 10^{-8}$. The assembly method used above was described in "System and Methods for Analyzing Biomolecular Sequences," U.S.S.N. 09/276,534, filed March 25, 1999, and the LIFESEQ Gold user manual (Incyte) both incorporated by reference herein.

Following assembly, template sequences were subjected to motif, BLAST, and functional analyses, and categorized in protein hierarchies using methods described in, e.g., "Database System Employing Protein Function Hierarchies for Viewing Biomolecular Sequence Data," U.S.S.N. 08/812,290, filed March 6, 1997; "Relational Database for Storing Biomolecule Information,"

U.S.S.N. 08/947,845, filed October 9, 1997; "Project-Based Full-Length Biomolecular Sequence Database," U.S.S.N. 08/811,758, filed March 6, 1997; and "Relational Database and System for Storing Information Relating to Biomolecular Sequences," U.S.S.N. 09/034,807, filed March 4, 1998, all of which are incorporated by reference herein.

5 The template sequences were further analyzed by translating each template in all three forward reading frames and searching each translation against the Pfam database of hidden Markov model-based protein families and domains using the HMMER software package (available to the public from Washington University School of Medicine, St. Louis MO). Regions of templates which, when translated, contain similarity to Pfam consensus sequences are reported in Table 2, along with
10 descriptions of Pfam protein domains and families. Only those Pfam hits with an E-value of $\leq 1 \times 10^{-3}$ are reported. (See also World Wide Web site <http://pfam.wustl.edu/> for detailed descriptions of Pfam protein domains and families.)

 Additionally, the template sequences were translated in all three forward reading frames, and each translation was searched against hidden Markov models for signal peptides using the HMMER
15 software package. Construction of hidden Markov models and their usage in sequence analysis has been described. (See, for example, Eddy, S.R. (1996) Curr. Opin. Str. Biol. 6:361-365.) Only those signal peptide hits with a cutoff score of 11 bits or greater are reported. A cutoff score of 11 bits or greater corresponds to at least about 91-94% true-positives in signal peptide prediction. Template sequences were also translated in all three forward reading frames, and each translation was searched
20 against TMAP, a program that uses weight matrices to delineate transmembrane segments on protein sequences and determine orientation, with respect to the cell cytosol (Persson, B. and P. Argos (1994) J. Mol. Biol. 237:182-192; Persson, B. and P. Argos (1996) Protein Sci. 5:363-371). Regions of templates which, when translated, contain similarity to signal peptide or transmembrane consensus sequences are reported in Table 3.

25 The results of HMMER analysis as reported in Tables 2 and 3 may support the results of BLAST analysis as reported in Table 1 or may suggest alternative or additional properties of template-encoded polypeptides not previously uncovered by BLAST or other analyses.

 Template sequences are further analyzed using the bioinformatics tools listed in Table 7, or using sequence analysis software known in the art such as MACDNASIS PRO software (Hitachi
30 Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Template sequences may be further queried against public databases such as the GenBank rodent, mammalian, vertebrate, prokaryote, and eukaryote databases.

 The template sequences were translated to derive the corresponding longest open reading frame as presented by the polypeptide sequences. Alternatively, a polypeptide of the invention may begin at

any of the methionine residues within the full length translated polypeptide. Polypeptide sequences were subsequently analyzed by querying against the GenBank protein database (GENPEPT, (GenBank version 121)). Full length polynucleotide sequences are also analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments are generated using default parameters specified by the CLUSTAL algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

Table 6 shows sequences with homology to the polypeptides of the invention as identified by BLAST analysis against the GenBank protein (GENPEPT) database. Column 1 shows the polypeptide sequence identification number (SEQ ID NO:) for the polypeptide segments of the invention. Column 2 shows the reading frame used in the translation of the polynucleotide sequences encoding the polypeptide segments. Column 3 shows the length of the translated polypeptide segments. Columns 4 and 5 show the start and stop nucleotide positions of the polynucleotide sequences encoding the polypeptide segments. Column 6 shows the GenBank identification number (GI Number) of the nearest GenBank homolog. Column 7 shows the probability score for the match between each polypeptide and its GenBank homolog. Column 8 shows the annotation of the GenBank homolog.

V. Analysis of Polynucleotide Expression

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, *supra*, ch. 7; Ausubel, 1995, *supra*, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in cDNA databases such as GenBank or LIFESEQ (Incyte Genomics). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\text{BLAST Score} \times \text{Percent Identity}}{5 \times \text{minimum \{length(Seq. 1), length(Seq. 2)\}}}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. The product score is a normalized value between 0 and 100, and is calculated as follows: the BLAST score is multiplied by the percent nucleotide identity and the product is divided by (5 times the length of the shorter of the two sequences). The BLAST score is calculated by

assigning a score of +5 for every base that matches in a high-scoring segment pair (HSP), and -4 for every mismatch. Two sequences may share more than one HSP (separated by gaps). If there is more than one HSP, then the pair with the highest BLAST score is used to calculate the product score. The product score represents a balance between fractional overlap and quality in a BLAST alignment. For example, a product score of 100 is produced only for 100% identity over the entire length of the shorter of the two sequences being compared. A product score of 70 is produced either by 100% identity and 70% overlap at one end, or by 88% identity and 100% overlap at the other. A product score of 50 is produced either by 100% identity and 50% overlap at one end, or 79% identity and 100% overlap.

10 VI. Tissue Distribution Profiling

A tissue distribution profile is determined for each template by compiling the cDNA library tissue classifications of its component cDNA sequences. Each component sequence, is derived from a cDNA library constructed from a human tissue. Each human tissue is classified into one of the following categories: cardiovascular system; connective tissue; digestive system; embryonic structures; endocrine system; exocrine glands; genitalia, female; genitalia, male; germ cells; hemic and immune system; liver; musculoskeletal system; nervous system; pancreas; respiratory system; sense organs; skin; stomatognathic system; unclassified/mixed; or urinary tract. Template sequences, component sequences, and cDNA library/tissue information are found in the LIFESEQ GOLD database (Incyte Genomics, Palo Alto CA).

20 Table 5 shows the tissue distribution profile for the templates of the invention. For each template, the three most frequently observed tissue categories are shown in column 3, along with the percentage of component sequences belonging to each category. Only tissue categories with percentage values of $\geq 10\%$ are shown. A tissue distribution of "widely distributed" in column 3 indicates percentage values of $<10\%$ in all tissue categories.

25

VII. Transcript Image Analysis

Transcript images are generated as described in Seilhamer et al., "Comparative Gene Transcript Analysis," U.S. Patent Number 5,840,484, incorporated herein by reference.

30 VIII. Extension of Polynucleotide Sequences and Isolation of a Full-length cDNA

Oligonucleotide primers designed using a dithp of the Sequence Listing are used to extend the nucleic acid sequence. One primer is synthesized to initiate 5' extension of the template, and the other primer, to initiate 3' extension of the template. The initial primers may be designed using OLIGO 4.06 software (National Biosciences, Inc. (National Biosciences), Plymouth MN), or another appropriate

program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations are avoided. Selected human cDNA libraries are used to extend the sequence. If more than one extension is necessary or desired,
5 additional or nested sets of primers are designed.

High fidelity amplification is obtained by PCR using methods well known in the art. PCR is performed in 96-well plates using the PTC-200 thermal cycler (MJ Research). The reaction mix contains DNA template, 200 nmol of each primer, reaction buffer containing Mg^{2+} , $(NH_4)_2SO_4$, and β -mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life
10 Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ are as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times;
15 Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well is determined by dispensing 100 μ l PICOGREEN quantitation reagent (0.25% (v/v); Molecular Probes) dissolved in 1X Tris-EDTA (TE) and 0.5 μ l of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Incorporated (Corning), Corning NY), allowing the DNA to bind to the reagent. The plate is scanned in a FLUOROSKAN II
20 (Labsystems Oy) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 μ l to 10 μ l aliquot of the reaction mixture is analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions are successful in extending the sequence.

The extended nucleotides are desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or
25 sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides are separated on low concentration (0.6 to 0.8%) agarose gels, fragments are excised, and agar digested with AGAR ACE (Promega). Extended clones are religated using T4 ligase (New England Biolabs, Inc., Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and
30 transfected into competent E. coli cells. Transformed cells are selected on antibiotic-containing media, individual colonies are picked and cultured overnight at 37°C in 384-well plates in LB/2x carbenicillin liquid media.

The cells are lysed, and DNA is amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1:

94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA is quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries are reamplified using the same conditions as described above. Samples are diluted with 20% dimethylsulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Applied Biosystems).

In like manner, the dithp is used to obtain regulatory sequences (promoters, introns, and enhancers) using the procedure above, oligonucleotides designed for such extension, and an appropriate genomic library.

IX. Labeling of Probes and Southern Hybridization Analyses

Hybridization probes derived from the dithp of the Sequence Listing are employed for screening cDNAs, mRNAs, or genomic DNA. The labeling of probe nucleotides between 100 and 1000 nucleotides in length is specifically described, but essentially the same procedure may be used with larger cDNA fragments. Probe sequences are labeled at room temperature for 30 minutes using a T4 polynucleotide kinase, $\gamma^{32}\text{P}$ -ATP, and 0.5X One-Phor-All Plus (Amersham Pharmacia Biotech) buffer and purified using a ProbeQuant G-50 Microcolumn (Amersham Pharmacia Biotech). The probe mixture is diluted to 10^7 dpm/ $\mu\text{g/ml}$ hybridization buffer and used in a typical membrane-based hybridization analysis.

The DNA is digested with a restriction endonuclease such as Eco RV and is electrophoresed through a 0.7% agarose gel. The DNA fragments are transferred from the agarose to nylon membrane (NYTRAN Plus, Schleicher & Schuell, Inc., Keene NH) using procedures specified by the manufacturer of the membrane. Prehybridization is carried out for three or more hours at 68°C, and hybridization is carried out overnight at 68°C. To remove non-specific signals, blots are sequentially washed at room temperature under increasingly stringent conditions, up to 0.1x saline sodium citrate (SSC) and 0.5% sodium dodecyl sulfate. After the blots are placed in a PHOSPHORIMAGER cassette (Molecular Dynamics) or are exposed to autoradiography film, hybridization patterns of standard and experimental lanes are compared. Essentially the same procedure is employed when screening RNA.

X. Chromosome Mapping of dithp

The cDNA sequences which were used to assemble SEQ ID NO:1-211 are compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that match SEQ

ID NO:1-211 are assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as PHRAP (Table 7). Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for Genome Research (WIGR), and Généthon are used to determine if any of the clustered sequences have been previously mapped. Inclusion of a mapped sequence in a cluster will result in the assignment of all sequences of that cluster, including its particular SEQ ID NO., to that map location. The genetic map locations of SEQ ID NO:1-211 are described as ranges, or intervals, of human chromosomes. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's p-arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters.

15 XI. Microarray Analysis

Probe Preparation from Tissue or Cell Samples

Total RNA is isolated from tissue samples using the guanidinium thiocyanate method and polyA⁺ RNA is purified using the oligo (dT) cellulose method. Each polyA⁺ RNA sample is reverse transcribed using MMLV reverse-transcriptase, 0.05 pg/ μ l oligo-dT primer (21mer), 1X first strand buffer, 0.03 units/ μ l RNase inhibitor, 500 μ M dATP, 500 μ M dGTP, 500 μ M dTTP, 40 μ M dCTP, 40 μ M dCTP-Cy3 (BDS) or dCTP-Cy5 (Amersham Pharmacia Biotech). The reverse transcription reaction is performed in a 25 ml volume containing 200 ng polyA⁺ RNA with GEMBRIGHT kits (Incyte). Specific control polyA⁺ RNAs are synthesized by *in vitro* transcription from non-coding yeast genomic DNA (W. Lei, unpublished). As quantitative controls, the control mRNAs at 0.002 ng, 0.02 ng, 0.2 ng, and 2 ng are diluted into reverse transcription reaction at ratios of 1:100,000, 1:10,000, 1:1000, 1:100 (w/w) to sample mRNA respectively. The control mRNAs are diluted into reverse transcription reaction at ratios of 1:3, 3:1, 1:10, 10:1, 1:25, 25:1 (w/w) to sample mRNA differential expression patterns. After incubation at 37°C for 2 hr, each reaction sample (one with Cy3 and another with Cy5 labeling) is treated with 2.5 ml of 0.5M sodium hydroxide and incubated for 20 minutes at 85°C to stop the reaction and degrade the RNA. Probes are purified using two successive CHROMA SPIN 30 gel filtration spin columns (CLONTECH Laboratories, Inc. (CLONTECH), Palo Alto CA) and after combining, both reaction samples are ethanol precipitated using 1 ml of glycogen (1 mg/ml), 60 ml sodium acetate, and 300 ml of 100% ethanol. The probe is then dried to completion using a SpeedVAC (Savant Instruments Inc., Holbrook NY) and resuspended in 14 μ l 5X SSC/0.2%

SDS.

Microarray Preparation

Sequences of the present invention are used to generate array elements. Each array element is amplified from bacterial cells containing vectors with cloned cDNA inserts. PCR amplification uses primers complementary to the vector sequences flanking the cDNA insert. Array elements are amplified in thirty cycles of PCR from an initial quantity of 1-2 ng to a final quantity greater than 5 µg. Amplified array elements are then purified using SEPHACRYL-400 (Amersham Pharmacia Biotech).

Purified array elements are immobilized on polymer-coated glass slides. Glass microscope slides (Corning) are cleaned by ultrasound in 0.1% SDS and acetone, with extensive distilled water washes between and after treatments. Glass slides are etched in 4% hydrofluoric acid (VWR Scientific Products Corporation (VWR), West Chester, PA), washed extensively in distilled water, and coated with 0.05% aminopropyl silane (Sigma) in 95% ethanol. Coated slides are cured in a 110°C oven.

Array elements are applied to the coated glass substrate using a procedure described in US Patent No. 5,807,522, incorporated herein by reference. 1 µl of the array element DNA, at an average concentration of 100 ng/µl, is loaded into the open capillary printing element by a high-speed robotic apparatus. The apparatus then deposits about 5 nl of array element sample per slide.

Microarrays are UV-crosslinked using a STRATALINKER UV-crosslinker (Stratagene). Microarrays are washed at room temperature once in 0.2% SDS and three times in distilled water. Non-specific binding sites are blocked by incubation of microarrays in 0.2% casein in phosphate buffered saline (PBS) (Tropix, Inc., Bedford, MA) for 30 minutes at 60° C followed by washes in 0.2% SDS and distilled water as before.

Hybridization

Hybridization reactions contain 9 µl of probe mixture consisting of 0.2 µg each of Cy3 and Cy5 labeled cDNA synthesis products in 5X SSC, 0.2% SDS hybridization buffer. The probe mixture is heated to 65° C for 5 minutes and is aliquoted onto the microarray surface and covered with an 1.8 cm² coverslip. The arrays are transferred to a waterproof chamber having a cavity just slightly larger than a microscope slide. The chamber is kept at 100% humidity internally by the addition of 140 µl of 5x SSC in a corner of the chamber. The chamber containing the arrays is incubated for about 6.5 hours at 60° C. The arrays are washed for 10 min at 45° C in a first wash buffer (1X SSC, 0.1% SDS), three times for 10 minutes each at 45° C in a second wash buffer (0.1X SSC), and dried.

Detection

Reporter-labeled hybridization complexes are detected with a microscope equipped with an Innova 70 mixed gas 10 W laser (Coherent, Inc., Santa Clara CA) capable of generating spectral lines at 488 nm for excitation of Cy3 and at 632 nm for excitation of Cy5. The excitation laser light is focused on the array using a 20X microscope objective (Nikon, Inc., Melville NY). The slide
5 containing the array is placed on a computer-controlled X-Y stage on the microscope and raster-scanned past the objective. The 1.8 cm x 1.8 cm array used in the present example is scanned with a resolution of 20 micrometers.

In two separate scans, a mixed gas multiline laser excites the two fluorophores sequentially. Emitted light is split, based on wavelength, into two photomultiplier tube detectors (PMT R1477,
10 Hamamatsu Photonics Systems, Bridgewater NJ) corresponding to the two fluorophores. Appropriate filters positioned between the array and the photomultiplier tubes are used to filter the signals. The emission maxima of the fluorophores used are 565 nm for Cy3 and 650 nm for Cy5. Each array is typically scanned twice, one scan per fluorophore using the appropriate filters at the laser source, although the apparatus is capable of recording the spectra from both fluorophores simultaneously.

The sensitivity of the scans is typically calibrated using the signal intensity generated by a cDNA control species added to the probe mix at a known concentration. A specific location on the array contains a complementary DNA sequence, allowing the intensity of the signal at that location to be correlated with a weight ratio of hybridizing species of 1:100,000. When two probes from different
15 sources (e.g., representing test and control cells), each labeled with a different fluorophore, are hybridized to a single array for the purpose of identifying genes that are differentially expressed, the calibration is done by labeling samples of the calibrating cDNA with the two fluorophores and adding identical amounts of each to the hybridization mixture.

The output of the photomultiplier tube is digitized using a 12-bit RTI-835H analog-to-digital (A/D) conversion board (Analog Devices, Inc., Norwood, MA) installed in an IBM-compatible PC
25 computer. The digitized data are displayed as an image where the signal intensity is mapped using a linear 20-color transformation to a pseudocolor scale ranging from blue (low signal) to red (high signal). The data is also analyzed quantitatively. Where two different fluorophores are excited and measured simultaneously, the data are first corrected for optical crosstalk (due to overlapping emission spectra) between the fluorophores using each fluorophore's emission spectrum.

A grid is superimposed over the fluorescence signal image such that the signal from each spot is centered in each element of the grid. The fluorescence signal within each element is then integrated to obtain a numerical value corresponding to the average intensity of the signal. The software used for
30 signal analysis is the GEMTOOLS gene expression analysis program (Incyte).

XII. Complementary Nucleic Acids

Sequences complementary to the dithp are used to detect, decrease, or inhibit expression of the naturally occurring nucleotide. The use of oligonucleotides comprising from about 15 to 30 base pairs is typical in the art. However, smaller or larger sequence fragments can also be used. Appropriate oligonucleotides are designed from the dithp using OLIGO 4.06 software (National Biosciences) or other appropriate programs and are synthesized using methods standard in the art or ordered from a commercial supplier. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent transcription factor binding to the promoter sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding and processing of the transcript.

XIII. Expression of DITHP

Expression and purification of DITHP is accomplished using bacterial or virus-based expression systems. For expression of DITHP in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express DITHP upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of DITHP in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding DITHP by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See e.g., Engelhard, supra; and Sandig, supra.)

In most expression systems, DITHP is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from DITHP at

specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak Company, Rochester NY). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in
5 Ausubel (1995, supra, Chapters 10 and 16). Purified DITHP obtained by these methods can be used directly in the following activity assay.

XIV. Demonstration of DITHP Activity

DITHP activity is demonstrated through a variety of specific assays, some of which are
10 outlined below.

Oxidoreductase activity of DITHP is measured by the increase in extinction coefficient of NAD(P)H coenzyme at 340 nm for the measurement of oxidation activity, or the decrease in extinction coefficient of NAD(P)H coenzyme at 340 nm for the measurement of reduction activity (Dalziel, K. (1963) J. Biol. Chem. 238:2850-2858). One of three substrates may be used: Asn- β Gal, biocytidine, or
15 ubiquinone-10. The respective subunits of the enzyme reaction, for example, cytochrome c_1 -b oxidoreductase and cytochrome c, are reconstituted. The reaction mixture contains a) 1-2 mg/ml DITHP; and b) 15 mM substrate, 2.4 mM NAD(P)⁺ in 0.1 M phosphate buffer, pH 7.1 (oxidation reaction), or 2.0 mM NAD(P)H, in 0.1 M Na₂HPO₄ buffer, pH 7.4 (reduction reaction); in a total volume of 0.1 ml. Changes in absorbance at 340 nm (A_{340}) are measured at 23.5° C using a recording
20 spectrophotometer (Shimadzu Scientific Instruments, Inc., Pleasanton CA). The amount of NAD(P)H is stoichiometrically equivalent to the amount of substrate initially present, and the change in A_{340} is a direct measure of the amount of NAD(P)H produced; $\Delta A_{340} = 6620[\text{NADH}]$. Oxidoreductase activity of DITHP activity is proportional to the amount of NAD(P)H present in the assay.

Transferase activity of DITHP is measured through assays such as a methyl transferase assay
25 in which the transfer of radiolabeled methyl groups between a donor substrate and an acceptor substrate is measured (Bokar, J.A. et al. (1994) J. Biol. Chem. 269:17697-17704). Reaction mixtures (50 μ l final volume) contain 15 mM HEPES, pH 7.9, 1.5 mM MgCl₂, 10 mM dithiothreitol, 3% polyvinylalcohol, 1.5 μ Ci [*methyl*-³H]AdoMet (0.375 μ M AdoMet) (DuPont-NEN), 0.6 μ g DITHP, and acceptor substrate (0.4 μ g [³⁵S]RNA or 6-mercaptopurine (6-MP) to 1 mM final concentration).
30 Reaction mixtures are incubated at 30°C for 30 minutes, then 65 °C for 5 minutes. The products are separated by chromatography or electrophoresis and the level of methyl transferase activity is determined by quantification of *methyl*-³H recovery.

DITHP hydrolase activity is measured by the hydrolysis of appropriate synthetic peptide substrates conjugated with various chromogenic molecules in which the degree of hydrolysis is

quantified by spectrophotometric (or fluorometric) absorption of the released chromophore. (Beynon, R.J. and J.S. Bond (1994) Proteolytic Enzymes: A Practical Approach, Oxford University Press, New York NY, pp. 25-55) Peptide substrates are designed according to the category of protease activity as endopeptidase (serine, cysteine, aspartic proteases), aminopeptidase (leucine aminopeptidase), or
5 carboxypeptidase (Carboxypeptidase A and B, procollagen C-proteinase).

DITHP isomerase activity such as peptidyl prolyl *cis/trans* isomerase activity can be assayed by an enzyme assay described by Rahfeld, J.U., et al. (1994) (FEBS Lett. 352: 180-184). The assay is performed at 10°C in 35 mM HEPES buffer, pH 7.8, containing chymotrypsin (0.5 mg/ml) and DITHP at a variety of concentrations. Under these assay conditions, the substrate, Suc-Ala-Xaa-Pro-
10 Phe-4-NA, is in equilibrium with respect to the prolyl bond, with 80-95% in *trans* and 5-20% in *cis* conformation. An aliquot (2 ul) of the substrate dissolved in dimethyl sulfoxide (10 mg/ml) is added to the reaction mixture described above. Only the *cis* isomer of the substrate is a substrate for cleavage by chymotrypsin. Thus, as the substrate is isomerized by DITHP, the product is cleaved by chymotrypsin to produce 4-nitroanilide, which is detected by its absorbance at 390 nm. 4-
15 Nitroanilide appears in a time-dependent and a DITHP concentration-dependent manner.

An assay for DITHP activity associated with growth and development measures cell proliferation as the amount of newly initiated DNA synthesis in Swiss mouse 3T3 cells. A plasmid containing polynucleotides encoding DITHP is transfected into quiescent 3T3 cultured cells using methods well known in the art. The transiently transfected cells are then incubated in the presence of
20 [³H]thymidine, a radioactive DNA precursor. Where applicable, varying amounts of DITHP ligand are added to the transfected cells. Incorporation of [³H]thymidine into acid-precipitable DNA is measured over an appropriate time interval, and the amount incorporated is directly proportional to the amount of newly synthesized DNA.

Growth factor activity of DITHP is measured by the stimulation of DNA synthesis in Swiss
25 mouse 3T3 cells (McKay, I. and I. Leigh, eds. (1993) Growth Factors: A Practical Approach, Oxford University Press, New York NY). Initiation of DNA synthesis indicates the cells' entry into the mitotic cycle and their commitment to undergo later division. 3T3 cells are competent to respond to most growth factors, not only those that are mitogenic, but also those that are involved in embryonic induction. This competence is possible because the *in vivo* specificity demonstrated by some growth
30 factors is not necessarily inherent but is determined by the responding tissue. In this assay, varying amounts of DITHP are added to quiescent 3T3 cultured cells in the presence of [³H]thymidine, a radioactive DNA precursor. DITHP for this assay can be obtained by recombinant means or from biochemical preparations. Incorporation of [³H]thymidine into acid-precipitable DNA is measured over an appropriate time interval, and the amount incorporated is directly proportional to the amount of

newly synthesized DNA. A linear dose-response curve over at least a hundred-fold DITHP concentration range is indicative of growth factor activity. One unit of activity per milliliter is defined as the concentration of DITHP producing a 50% response level, where 100% represents maximal incorporation of [³H]thymidine into acid-precipitable DNA.

5 Alternatively, an assay for cytokine activity of DITHP measures the proliferation of leukocytes. In this assay, the amount of tritiated thymidine incorporated into newly synthesized DNA is used to estimate proliferative activity. Varying amounts of DITHP are added to cultured leukocytes, such as granulocytes, monocytes, or lymphocytes, in the presence of [³H]thymidine, a radioactive DNA precursor. DITHP for this assay can be obtained by recombinant means or from
10 biochemical preparations. Incorporation of [³H]thymidine into acid-precipitable DNA is measured over an appropriate time interval, and the amount incorporated is directly proportional to the amount of newly synthesized DNA. A linear dose-response curve over at least a hundred-fold DITHP concentration range is indicative of DITHP activity. One unit of activity per milliliter is conventionally defined as the concentration of DITHP producing a 50% response level, where 100%
15 represents maximal incorporation of [³H]thymidine into acid-precipitable DNA.

 An alternative assay for DITHP cytokine activity utilizes a Boyden micro chamber (Neuroprobe, Cabin John MD) to measure leukocyte chemotaxis (Vicari, *supra*). In this assay, about 10⁵ migratory cells such as macrophages or monocytes are placed in cell culture media in the upper compartment of the chamber. Varying dilutions of DITHP are placed in the lower compartment. The
20 two compartments are separated by a 5 or 8 micron pore polycarbonate filter (Nucleopore, Pleasanton CA). After incubation at 37°C for 80 to 120 minutes, the filters are fixed in methanol and stained with appropriate labeling agents. Cells which migrate to the other side of the filter are counted using standard microscopy. The chemotactic index is calculated by dividing the number of migratory cells counted when DITHP is present in the lower compartment by the number of migratory cells counted
25 when only media is present in the lower compartment. The chemotactic index is proportional to the activity of DITHP.

 Alternatively, cell lines or tissues transformed with a vector containing dithp can be assayed for DITHP activity by immunoblotting. Cells are denatured in SDS in the presence of β-mercaptoethanol, nucleic acids removed by ethanol precipitation, and proteins purified by acetone precipitation. Pellets
30 are resuspended in 20 mM tris buffer at pH 7.5 and incubated with Protein G-Sepharose pre-coated with an antibody specific for DITHP. After washing, the Sepharose beads are boiled in electrophoresis sample buffer, and the eluted proteins subjected to SDS-PAGE. The SDS-PAGE is transferred to a nitrocellulose membrane for immunoblotting, and the DITHP activity is assessed by visualizing and quantifying bands on the blot using the antibody specific for DITHP as the primary antibody and ¹²⁵I-

labeled IgG specific for the primary antibody as the secondary antibody.

DITHP kinase activity is measured by phosphorylation of a protein substrate using γ -labeled [32 P]-ATP and quantitation of the incorporated radioactivity using a radioisotope counter. DITHP is incubated with the protein substrate, [32 P]-ATP, and an appropriate kinase buffer. The [32 P] incorporated into the product is separated from free [32 P]-ATP by electrophoresis and the incorporated [32 P] is counted. The amount of [32 P] recovered is proportional to the kinase activity of DITHP in the assay. A determination of the specific amino acid residue phosphorylated is made by phosphoamino acid analysis of the hydrolyzed protein.

In the alternative, DITHP activity is measured by the increase in cell proliferation resulting from transformation of a mammalian cell line such as COS7, HeLa or CHO with an eukaryotic expression vector encoding DITHP. Eukaryotic expression vectors are commercially available, and the techniques to introduce them into cells are well known to those skilled in the art. The cells are incubated for 48-72 hours after transformation under conditions appropriate for the cell line to allow expression of DITHP. Phase microscopy is then used to compare the mitotic index of transformed versus control cells. An increase in the mitotic index indicates DITHP activity.

In a further alternative, an assay for DITHP signaling activity is based upon the ability of GPCR family proteins to modulate G protein-activated second messenger signal transduction pathways (e.g., cAMP; Gaudin, P. et al. (1998) J. Biol. Chem. 273:4990-4996). A plasmid encoding full length DITHP is transfected into a mammalian cell line (e.g., Chinese hamster ovary (CHO) or human embryonic kidney (HEK-293) cell lines) using methods well-known in the art. Transfected cells are grown in 12-well trays in culture medium for 48 hours, then the culture medium is discarded, and the attached cells are gently washed with PBS. The cells are then incubated in culture medium with or without ligand for 30 minutes, then the medium is removed and cells lysed by treatment with 1 M perchloric acid. The cAMP levels in the lysate are measured by radioimmunoassay using methods well-known in the art. Changes in the levels of cAMP in the lysate from cells exposed to ligand compared to those without ligand are proportional to the amount of DITHP present in the transfected cells.

Alternatively, an assay for DITHP protein phosphatase activity measures the hydrolysis of P-nitrophenyl phosphate (PNPP). DITHP is incubated together with PNPP in HEPES buffer pH 7.5, in the presence of 0.1% β -mercaptoethanol at 37°C for 60 min. The reaction is stopped by the addition of 6 ml of 10 N NaOH, and the increase in light absorbance of the reaction mixture at 410 nm resulting from the hydrolysis of PNPP is measured using a spectrophotometer. The increase in light absorbance is proportional to the phosphatase activity of DITHP in the assay (Diamond, R.H. et al (1994) Mol Cell Biol 14:3752-3762).

An alternative assay measures DITHP-mediated G-protein signaling activity by monitoring the mobilization of Ca^{++} as an indicator of the signal transduction pathway stimulation. (See, e.g., Grynkievicz, G. et al. (1985) J. Biol. Chem. 260:3440; McColl, S. et al. (1993) J. Immunol. 150:4550-4555; and Aussel, C. et al. (1988) J. Immunol. 140:215-220). The assay requires
5 preloading neutrophils or T cells with a fluorescent dye such as FURA-2 or BCECF (Universal Imaging Corp, Westchester PA) whose emission characteristics are altered by Ca^{++} binding. When the cells are exposed to one or more activating stimuli artificially (e.g., anti-CD3 antibody ligation of the T cell receptor) or physiologically (e.g., by allogeneic stimulation), Ca^{++} flux takes place. This flux can be observed and quantified by assaying the cells in a fluorometer or fluorescent activated cell
10 sorter. Measurements of Ca^{++} flux are compared between cells in their normal state and those transfected with DITHP. Increased Ca^{++} mobilization attributable to increased DITHP concentration is proportional to DITHP activity.

DITHP transport activity is assayed by measuring uptake of labeled substrates into Xenopus laevis oocytes. Oocytes at stages V and VI are injected with DITHP mRNA (10 ng per oocyte) and
15 incubated for 3 days at 18°C in OR2 medium (82.5mM NaCl, 2.5 mM KCl, 1mM CaCl_2 , 1mM MgCl_2 , 1mM Na_2HPO_4 , 5 mM Hepes, 3.8 mM NaOH, 50µg/ml gentamycin, pH 7.8) to allow expression of DITHP protein. Oocytes are then transferred to standard uptake medium (100mM NaCl, 2 mM KCl, 1mM CaCl_2 , 1mM MgCl_2 , 10 mM Hepes/Tris pH 7.5). Uptake of various substrates (e.g., amino acids, sugars, drugs, ions, and neurotransmitters) is initiated by adding labeled substrate (e.g.
20 radiolabeled with ^3H , fluorescently labeled with rhodamine, etc.) to the oocytes. After incubating for 30 minutes, uptake is terminated by washing the oocytes three times in Na^+ -free medium, measuring the incorporated label, and comparing with controls. DITHP transport activity is proportional to the level of internalized labeled substrate.

DITHP transferase activity is demonstrated by a test for galactosyltransferase activity. This
25 can be determined by measuring the transfer of radiolabeled galactose from UDP-galactose to a GlcNAc-terminated oligosaccharide chain (Kolbinger, F. et al. (1998) J. Biol. Chem. 273:58-65). The sample is incubated with 14 µl of assay stock solution (180 mM sodium cacodylate, pH 6.5, 1 mg/ml bovine serum albumin, 0.26 mM UDP-galactose, 2 µl of UDP- ^3H galactose), 1 µl of MnCl_2 (500 mM), and 2.5 µl of $\text{GlcNAc}\beta\text{O}-(\text{CH}_2)_8-\text{CO}_2\text{Me}$ (37 mg/ml in dimethyl sulfoxide) for 60 minutes at
30 37°C. The reaction is quenched by the addition of 1 ml of water and loaded on a C18 Sep-Pak cartridge (Waters), and the column is washed twice with 5 ml of water to remove unreacted UDP- ^3H galactose. The ^3H galactosylated $\text{GlcNAc}\beta\text{O}-(\text{CH}_2)_8-\text{CO}_2\text{Me}$ remains bound to the column during the water washes and is eluted with 5 ml of methanol. Radioactivity in the eluted material is measured by liquid scintillation counting and is proportional to galactosyltransferase activity in the starting

sample.

In the alternative, DITHP induction by heat or toxins may be demonstrated using primary cultures of human fibroblasts or human cell lines such as CCL-13, HEK293, or HEP G2 (ATCC). To heat induce DITHP expression, aliquots of cells are incubated at 42 °C for 15, 30, or 60 minutes.

- 5 Control aliquots are incubated at 37 °C for the same time periods. To induce DITHP expression by toxins, aliquots of cells are treated with 100 µM arsenite or 20 mM azetidine-2-carboxylic acid for 0, 3, 6, or 12 hours. After exposure to heat, arsenite, or the amino acid analogue, samples of the treated cells are harvested and cell lysates prepared for analysis by western blot. Cells are lysed in lysis buffer containing 1% Nonidet P-40, 0.15 M NaCl, 50 mM Tris-HCl, 5 mM EDTA, 2 mM
- 10 N-ethylmaleimide, 2 mM phenylmethylsulfonyl fluoride, 1 mg/ml leupeptin, and 1 mg/ml pepstatin. Twenty micrograms of the cell lysate is separated on an 8% SDS-PAGE gel and transferred to a membrane. After blocking with 5% nonfat dry milk/phosphate-buffered saline for 1 h, the membrane is incubated overnight at 4°C or at room temperature for 2-4 hours with a 1:1000 dilution of anti-DITHP serum in 2% nonfat dry milk/phosphate-buffered saline. The membrane is then washed
- 15 and incubated with a 1:1000 dilution of horseradish peroxidase-conjugated goat anti-rabbit IgG in 2% dry milk/phosphate-buffered saline. After washing with 0.1% Tween 20 in phosphate-buffered saline, the DITHP protein is detected and compared to controls using chemiluminescence.

- Alternatively, DITHP protease activity is measured by the hydrolysis of appropriate synthetic peptide substrates conjugated with various chromogenic molecules in which the degree of hydrolysis
- 20 is quantified by spectrophotometric (or fluorometric) absorption of the released chromophore (Beynon, R.J. and J.S. Bond (1994) Proteolytic Enzymes: A Practical Approach, Oxford University Press, New York, NY, pp.25-55). Peptide substrates are designed according to the category of protease activity as endopeptidase (serine, cysteine, aspartic proteases, or metalloproteases), aminopeptidase (leucine aminopeptidase), or carboxypeptidase (carboxypeptidases A and B,
- 25 procollagen C-proteinase). Commonly used chromogens are 2-naphthylamine, 4-nitroaniline, and furylacrylic acid. Assays are performed at ambient temperature and contain an aliquot of the enzyme and the appropriate substrate in a suitable buffer. Reactions are carried out in an optical cuvette, and the increase/decrease in absorbance of the chromogen released during hydrolysis of the peptide substrate is measured. The change in absorbance is proportional to the DITHP protease activity in the
- 30 assay.

In the alternative, an assay for DITHP protease activity takes advantage of fluorescence resonance energy transfer (FRET) that occurs when one donor and one acceptor fluorophore with an appropriate spectral overlap are in close proximity. A flexible peptide linker containing a cleavage site specific for PRTS is fused between a red-shifted variant (RSGFP4) and a blue variant (BFP5) of

Green Fluorescent Protein. This fusion protein has spectral properties that suggest energy transfer is occurring from BFP5 to RSGFP4. When the fusion protein is incubated with DITHP, the substrate is cleaved, and the two fluorescent proteins dissociate. This is accompanied by a marked decrease in energy transfer which is quantified by comparing the emission spectra before and after the addition of DITHP (Mitra, R.D. et al (1996) Gene 173:13-17). This assay can also be performed in living cells. In this case the fluorescent substrate protein is expressed constitutively in cells and DITHP is introduced on an inducible vector so that FRET can be monitored in the presence and absence of DITHP (Sagot, I. et al (1999) FEBS Lett. 447:53-57).

A method to determine the nucleic acid binding activity of DITHP involves a polyacrylamide gel mobility-shift assay. In preparation for this assay, DITHP is expressed by transforming a mammalian cell line such as COS7, HeLa or CHO with a eukaryotic expression vector containing DITHP cDNA. The cells are incubated for 48-72 hours after transformation under conditions appropriate for the cell line to allow expression and accumulation of DITHP. Extracts containing solubilized proteins can be prepared from cells expressing DITHP by methods well known in the art. Portions of the extract containing DITHP are added to [³²P]-labeled RNA or DNA. Radioactive nucleic acid can be synthesized in vitro by techniques well known in the art. The mixtures are incubated at 25 °C in the presence of RNase- and DNase-inhibitors under buffered conditions for 5-10 minutes. After incubation, the samples are analyzed by polyacrylamide gel electrophoresis followed by autoradiography. The presence of a band on the autoradiogram indicates the formation of a complex between DITHP and the radioactive transcript. A band of similar mobility will not be present in samples prepared using control extracts prepared from untransformed cells.

In the alternative, a method to determine the methylase activity of a DITHP measures transfer of radiolabeled methyl groups between a donor substrate and an acceptor substrate. Reaction mixtures (50 µl final volume) contain 15 mM HEPES, pH 7.9, 1.5 mM MgCl₂, 10 mM dithiothreitol, 3% polyvinylalcohol, 1.5 µCi [*methyl*-³H]AdoMet (0.375 µM AdoMet) (DuPont-NEN), 0.6 µg DITHP, and acceptor substrate (e.g., 0.4 µg [³⁵S]RNA, or 6-mercaptopurine (6-MP) to 1 mM final concentration). Reaction mixtures are incubated at 30 °C for 30 minutes, then 65 °C for 5 minutes. Analysis of [*methyl*-³H]RNA is as follows: 1) 50 µl of 2 x loading buffer (20 mM Tris-HCl, pH 7.6, 1 M LiCl, 1 mM EDTA, 1% sodium dodecyl sulphate (SDS)) and 50 µl oligo d(T)-cellulose (10 mg/ml in 1 x loading buffer) are added to the reaction mixture, and incubated at ambient temperature with shaking for 30 minutes. 2) Reaction mixtures are transferred to a 96-well filtration plate attached to a vacuum apparatus. 3) Each sample is washed sequentially with three 2.4 ml aliquots of 1 x oligo d(T) loading buffer containing 0.5% SDS, 0.1% SDS, or no SDS. and 4) RNA is eluted with 300 µl of water into a 96-well collection plate, transferred to scintillation vials containing liquid scintillant, and

radioactivity determined. Analysis of [methyl-³H]6-MP is as follows: 1) 500 µl 0.5 M borate buffer, pH 10.0, and then 2.5 ml of 20% (v/v) isoamyl alcohol in toluene are added to the reaction mixtures. 2) The samples mixed by vigorous vortexing for ten seconds. 3) After centrifugation at 700g for 10 minutes, 1.5 ml of the organic phase is transferred to scintillation vials containing 0.5 ml absolute ethanol and liquid scintillant, and radioactivity determined. and 4) Results are corrected for the extraction of 6-MP into the organic phase (approximately 41%).

An assay for adhesion activity of DITHP measures the disruption of cytoskeletal filament networks upon overexpression of DITHP in cultured cell lines (Reznicek, G.A. et al. (1998) J. Cell Biol. 141:209-225). cDNA encoding DITHP is subcloned into a mammalian expression vector that drives high levels of cDNA expression. This construct is transfected into cultured cells, such as rat kangaroo PtK2 or rat bladder carcinoma 804G cells. Actin filaments and intermediate filaments such as keratin and vimentin are visualized by immunofluorescence microscopy using antibodies and techniques well known in the art. The configuration and abundance of cytoskeletal filaments can be assessed and quantified using confocal imaging techniques. In particular, the bundling and collapse of cytoskeletal filament networks is indicative of DITHP adhesion activity.

Alternatively, an assay for DITHP activity measures the expression of DITHP on the cell surface. cDNA encoding DITHP is transfected into a non-leukocytic cell line. Cell surface proteins are labeled with biotin (de la Fuente, M.A. et al. (1997) Blood 90:2398-2405). Immunoprecipitations are performed using DITHP-specific antibodies, and immunoprecipitated samples are analyzed using SDS-PAGE and immunoblotting techniques. The ratio of labeled immunoprecipitant to unlabeled immunoprecipitant is proportional to the amount of DITHP expressed on the cell surface.

Alternatively, an assay for DITHP activity measures the amount of cell aggregation induced by overexpression of DITHP. In this assay, cultured cells such as NIH3T3 are transfected with cDNA encoding DITHP contained within a suitable mammalian expression vector under control of a strong promoter. Cotransfection with cDNA encoding a fluorescent marker protein, such as Green Fluorescent Protein (CLONTECH), is useful for identifying stable transfectants. The amount of cell agglutination, or clumping, associated with transfected cells is compared with that associated with untransfected cells. The amount of cell agglutination is a direct measure of DITHP activity.

DITHP may recognize and precipitate antigen from serum. This activity can be measured by the quantitative precipitin reaction (Golub, E.S. et al. (1987) Immunology: A Synthesis, Sinauer Associates, Sunderland MA, pages 113-115). DITHP is isotopically labeled using methods known in the art. Various serum concentrations are added to constant amounts of labeled DITHP. DITHP-antigen complexes precipitate out of solution and are collected by centrifugation. The amount of precipitable DITHP-antigen complex is proportional to the amount of radioisotope detected in the

precipitate. The amount of precipitable DITHP-antigen complex is plotted against the serum concentration. For various serum concentrations, a characteristic precipitation curve is obtained, in which the amount of precipitable DITHP-antigen complex initially increases proportionately with increasing serum concentration, peaks at the equivalence point, and then decreases proportionately with further increases in serum concentration. Thus, the amount of precipitable DITHP-antigen complex is a measure of DITHP activity which is characterized by sensitivity to both limiting and excess quantities of antigen.

A microtubule motility assay for DITHP measures motor protein activity. In this assay, recombinant DITHP is immobilized onto a glass slide or similar substrate. Taxol-stabilized bovine brain microtubules (commercially available) in a solution containing ATP and cytosolic extract are perfused onto the slide. Movement of microtubules as driven by DITHP motor activity can be visualized and quantified using video-enhanced light microscopy and image analysis techniques. DITHP motor protein activity is directly proportional to the frequency and velocity of microtubule movement.

Alternatively, an assay for DITHP measures the formation of protein filaments in vitro. A solution of DITHP at a concentration greater than the "critical concentration" for polymer assembly is applied to carbon-coated grids. Appropriate nucleation sites may be supplied in the solution. The grids are negative stained with 0.7% (w/v) aqueous uranyl acetate and examined by electron microscopy. The appearance of filaments of approximately 25 nm (microtubules), 8 nm (actin), or 10 nm (intermediate filaments) is a demonstration of protein activity.

DITHP electron transfer activity is demonstrated by oxidation or reduction of NADP. Substrates such as Asn- β Gal, biocytidine, or ubiquinone-10 may be used. The reaction mixture contains 1-2 mg/ml HORP, 15 mM substrate, and 2.4 mM NAD(P)⁺ in 0.1 M phosphate buffer, pH 7.1 (oxidation reaction), or 2.0 mM NAD(P)H, in 0.1 M Na₂HPO₄ buffer, pH 7.4 (reduction reaction); in a total volume of 0.1 ml. FAD may be included with NAD, according to methods well known in the art. Changes in absorbance are measured using a recording spectrophotometer. The amount of NAD(P)H is stoichiometrically equivalent to the amount of substrate initially present, and the change in A₃₄₀ is a direct measure of the amount of NAD(P)H produced; $\Delta A_{340} = 6620[\text{NADH}]$. DITHP activity is proportional to the amount of NAD(P)H present in the assay. The increase in extinction coefficient of NAD(P)H coenzyme at 340 nm is a measure of oxidation activity, or the decrease in extinction coefficient of NAD(P)H coenzyme at 340 nm is a measure of reduction activity (Dalziel, K. (1963) J. Biol. Chem. 238:2850-2858).

DITHP transcription factor activity is measured by its ability to stimulate transcription of a reporter gene (Liu, H.Y. et al. (1997) EMBO J. 16:5289-5298). The assay entails the use of a well

characterized reporter gene construct, LexA_{op}-LacZ, that consists of LexA DNA transcriptional control elements (LexA_{op}) fused to sequences encoding the *E. coli* LacZ enzyme. The methods for constructing and expressing fusion genes, introducing them into cells, and measuring LacZ enzyme activity, are well known to those skilled in the art. Sequences encoding DITHP are cloned into a plasmid that directs the synthesis of a fusion protein, LexA-DITHP, consisting of DITHP and a DNA binding domain derived from the LexA transcription factor. The resulting plasmid, encoding a LexA-DITHP fusion protein, is introduced into yeast cells along with a plasmid containing the LexA_{op}-LacZ reporter gene. The amount of LacZ enzyme activity associated with LexA-DITHP transfected cells, relative to control cells, is proportional to the amount of transcription stimulated by the DITHP.

Chromatin activity of DITHP is demonstrated by measuring sensitivity to DNase I (Dawson, B.A. et al. (1989) J. Biol. Chem. 264:12830-12837). Samples are treated with DNase I, followed by insertion of a cleavable biotinylated nucleotide analog, 5-[(N-biotinamido)hexanoamido-ethyl-1,3-thiopropionyl-3-aminoallyl]-2'-deoxyuridine 5'-triphosphate using nick-repair techniques well known to those skilled in the art. Following purification and digestion with EcoRI restriction endonuclease, biotinylated sequences are affinity isolated by sequential binding to streptavidin and biotincellulose.

Another specific assay demonstrates the ion conductance capacity of DITHP using an electrophysiological assay. DITHP is expressed by transforming a mammalian cell line such as COS7, HeLa or CHO with a eukaryotic expression vector encoding DITHP. Eukaryotic expression vectors are commercially available, and the techniques to introduce them into cells are well known to those skilled in the art. A small amount of a second plasmid, which expresses any one of a number of marker genes such as β -galactosidase, is co-transformed into the cells in order to allow rapid identification of those cells which have taken up and expressed the foreign DNA. The cells are incubated for 48-72 hours after transformation under conditions appropriate for the cell line to allow expression and accumulation of DITHP and β -galactosidase. Transformed cells expressing β -galactosidase are stained blue when a suitable colorimetric substrate is added to the culture media under conditions that are well known in the art. Stained cells are tested for differences in membrane conductance due to various ions by electrophysiological techniques that are well known in the art. Untransformed cells, and/or cells transformed with either vector sequences alone or β -galactosidase sequences alone, are used as controls and tested in parallel. The contribution of DITHP to cation or anion conductance can be shown by incubating the cells using antibodies specific for either DITHP. The respective antibodies will bind to the extracellular side of DITHP, thereby blocking the pore in the ion channel, and the associated conductance.

XV. Functional Assays

DITHP function is assessed by expressing dithp at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen Corporation, Carlsbad CA), both of which contain the
5 cytomagalovirus promoter. 5-10 µg of recombinant vector are transiently transfected into a human cell line, preferably of endothelial or hematopoietic origin, using either liposome formulations or electroporation. 1-2 µg of an additional plasmid containing sequences encoding a marker protein are co-transfected.

Expression of a marker protein provides a means to distinguish transfected cells from
10 nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; CLONTECH), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties.

15 FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular
20 proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M. G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of DITHP on gene expression can be assessed using highly purified populations
25 of cells transfected with sequences encoding DITHP and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Inc., Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art.
30 Expression of mRNA encoding DITHP and other genes of interest can be analyzed by northern analysis or microarray techniques.

XVI. Production of Antibodies

DITHP substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g.,

Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the DITHP amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding peptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, *supra*, Chapter 11.)

Typically, peptides 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Applied Biosystems) using fmoc-chemistry and coupled to KLH (Sigma) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, *supra*.) Rabbits are immunized with the peptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for anti-peptide activity by, for example, binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radioiodinated goat anti-rabbit IgG. Antisera with anti-peptide activity are tested for anti-DITHP activity using protocols well known in the art, including ELISA, RIA, and immunoblotting.

XVII. Purification of Naturally Occurring DITHP Using Specific Antibodies

Naturally occurring or recombinant DITHP is substantially purified by immunoaffinity chromatography using antibodies specific for DITHP. An immunoaffinity column is constructed by covalently coupling anti-DITHP antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing DITHP are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of DITHP (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/DITHP binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and DITHP is collected.

XVIII. Identification of Molecules Which Interact with DITHP

DITHP, or biologically active fragments thereof, are labeled with ^{125}I Bolton-Hunter reagent. (See, e.g., Bolton, A.E. and W.M. Hunter (1973) *Biochem. J.* 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled DITHP, washed, and any wells with labeled DITHP complex are assayed. Data obtained using different concentrations of DITHP are used to calculate values for the number, affinity, and association of DITHP with the

candidate molecules.

Alternatively, molecules interacting with DITHP are analyzed using the yeast two-hybrid system as described in Fields, S. and O. Song (1989) Nature 340:245-246, or using commercially available kits based on the two-hybrid system, such as the MATCHMAKER system (CLONTECH).

5 DITHP may also be used in the PATHCALLING process (CuraGen Corp., New Haven CT) which employs the yeast two-hybrid system in a high-throughput manner to determine all interactions between the proteins encoded by two large libraries of genes (Nandabalan, K. et al. (2000) U.S. Patent No. 6,057,101).

10 All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments.

15 Indeed, various modifications of the above-described modes for carrying out the invention which are obvious to those skilled in the field of molecular biology or related fields are intended to be within the scope of the following claims.

TABLE 1

SEQ ID NO:	Template ID	GI Number	Probability Score	Annotation
1	LG:1040582.1:2000FEB18	g178480	1.00E-92	Human aldehyde reductase mRNA, complete cds.
2	LG:453570.1:2000FEB18	g2909424	2.40E-65	Glyoxalase I
3	LG:408751.3:2000FEB18	g3608122	4.40E-74	dihydropyrimidinase
4	LI:090574.1:2000FEB01	g296600	5.00E-85	carbonic anhydrase I (AA 1-261)
5	LI:229932.2:2000FEB01	g1835116	2.00E-63	acetyl-CoA synthetase
6	LI:332176.1:2000FEB01	g2104689	0	alpha glucosidase II, alpha subunit
7	LI:403248.2:2000FEB01	g63713	4.00E-23	ornithine decarboxylase
8	LG:220992.1:2000MAY19	g10435462	0	unnamed protein product (Homo sapiens)
9	LG:1094571.1:2000MAY19	g7023634	4.00E-92	unnamed protein product (Homo sapiens)
10	LI:350754.4:2000MAY01	g307504	0	transglutaminase E3 (Homo sapiens)
11	LI:255828.29:2000MAY01	g189998	9.00E-65	M2-type pyruvate kinase (Homo sapiens)
12	LI:1190263.1:2000MAY01	g2576305	1.00E-172	arylsulphatase (Homo sapiens)
13	LG:270916.2:2000FEB18	g2088668	1.20E-11	similar to Achlya ambisexualis antheridial steroid receptor (NID:g166306)
14	LG:999414.3:2000FEB18	g3861482	0	Human chromosome 3, olfactory receptor pseudogene cluster 1, complete sequence, and myosin light chain kinase (MLCK)
15	LG:429446.1:2000FEB18	g2358042	0	pseudogene, partial sequence.
16	LI:057229.1:2000FEB01	g10439739	2.00E-19	Human T-cell receptor alpha delta locus from bases 501613 to 752736 (section 3 of 5) of the Complete Nucleotide Sequence.
17	LI:351965.1:2000FEB01	g2358042	0	unnamed protein product (Homo sapiens)
18	LG:086682.1:2000FEB18	g404634	1.10E-31	Human T-cell receptor alpha delta locus from bases 501613 to 752736 (section 3 of 5) of the Complete Nucleotide Sequence.
19	LG:242665.1:2000FEB18	g2117166	1.00E-160	serine/threonine kinase
20	LG:241743.1:2000FEB18	g5763838	9.70E-49	Ras like GTPase (Homo sapiens)
21	LI:034212.1:2000FEB01	g1469876	0	dJ593C16.1 (ras GTPase activating protein)
22	LG:344886.1:2000MAY19	g7008402	2.00E-89	The KIAA0147 gene product is related to adenyl cyclase.
23	LG:228930.1:2000MAY19	g206218	4.50E-87	kappa B-ras 1 (Homo sapiens)
24	LG:338927.1:2000MAY19	g3599940	3.00E-57	phospholipase C-1
25	LG:898771.1:2000MAY19	g508528	5.00E-58	faciogenital dysplasia protein 2 (Mus musculus)
26	LI:257664.67:2000MAY01	g183399	1.00E-142	myocyte nuclear factor (Mus musculus)
27	LI:001496.2:2000MAY01	g3005085	1.00E-177	Human guanine nucleotide-binding protein alpha-subunit gene (G-s-alpha), exon 3.
28	LI:1085273.2:2000MAY01	g1781037	0	hook1 protein (Homo sapiens)
29	LI:333138.2:2000MAY01	g2077934	1.00E-164	neuronal tyrosine threonine phosphatase 1 (Mus musculus)

30	LI:338927.1:2000MAY01	g3599940	6.00E-45	faciogenital dysplasia protein 2 (Mus musculus)
31	LG:335558.1:2000FEB18	g1181619	5.00E-97	a variant of TSC-22 (Gallus gallus)
32	LG:998283.7:2000FEB18	g6683492	1.00E-105	bromodomain PHD finger transcription factor (Homo sapiens)
33	LI:402739.1:2000FEB01	g4164151	6.00E-34	Ahr repressor
34	LI:175223.1:2000FEB01	g2745892	2.00E-11	Y box transcription factor
35	LG:981076.2:2000MAY19	g3924670	1.00E-59	supported by Genscan and several ESTs: C83049 (NID:g3062006),
36	LI:1008973.1:2000MAY01	g6939732	2.00E-52	AA823760 (NID:g2893628), AA215791 (NID:g1815572), A1095488
37	LI:1190250.1:2000MAY01	g3757892	4.00E-66	(NID:a3434464), and AA96095 (NID:g314275) (Homo sapiens)
38	LG:021371.3:2000FEB18	g984814	1.40E-60	transcription factor Elongin A2 (Homo sapiens)
39	LG:475404.1:2000FEB18	g487784	5.00E-36	enhancer of polycarb (Mus musculus)
40	LG:979406.2:2000FEB18	g4325310	3.60E-11	zinc finger protein
41	LG:410726.1:2000FEB18	g6002480	2.60E-39	Human zinc finger protein ZNF136.
42	LG:200005.1:2000FEB18	g1504006	3.20E-25	zinc-finger protein 7
43	LG:1076828.1:2000FEB18	g498720	2.00E-33	BWSCR2 associated zinc-finger protein BAZ2
44	LG:1076931.1:2000FEB18	g498151	2.00E-52	similarto human ZFY protein.
45	LG:1078121.1:2000FEB18	g186773	2.00E-47	Human HZF10 mRNA for zinc finger protein.
46	LG:1079203.1:2000FEB18	g1017721	0	Human mRNA for KIAA0065 gene, partial cds.
47	LG:1082586.1:2000FEB18	g7959207	1.00E-19	Human Kruppel related zinc finger protein (HNF10) mRNA, complete cds.
48	LG:1082774.1:2000FEB18	g184451	0	Human repressor transcriptional factor (ZNF85) mRNA, complete cds.
49	LG:1082775.1:2000FEB18	g506502	3.50E-36	KIAA1473 protein (Homo sapiens)
50	LG:1083120.1:2000FEB18	g7023216	1.00E-14	Human Krueppel-related DNA-binding protein (TF9 PF4) mRNA, 5' cds.
51	LG:1087707.1:2000FEB18	g347905	2.00E-40	NK10
52	LG:1090915.1:2000FEB18	g347905	2.00E-24	unnamed protein product (Homo sapiens)
53	LG:1094230.1:2000FEB18	g454818	1.00E-98	Human zinc finger protein (ZNF141) mRNA, complete cds.
54	LG:474848.3:2000FEB18	g498152	4.00E-16	Human zinc finger protein (ZNF141) mRNA, complete cds.
55	LI:251656.1:2000FEB01	g55471	0	Human Krueppel-related DNA-binding protein (PF4) mRNA, 5' end.
56	LI:021371.1:2000FEB01	g984814	2.00E-96	ha0946 protein is Kruppel-related.
57	LI:133095.1:2000FEB01	g453376	8.00E-42	Zfp-29
58	LI:236654.2:2000FEB01	g498721	2.00E-22	zinc finger protein
59	LI:200009.1:2000FEB01	g498719	4.00E-24	zinc finger protein
60	LI:758502.1:2000FEB01	g200407	0	pMLZ-4
61	LI:344772.1:2000FEB01	g4062983	3.00E-67	Eos protein
62	LI:789445.1:2000FEB01	g1049301	2.00E-26	KRAB zinc finger protein; Method: conceptual translation supplied by
63	LI:789657.1:2000FEB01	g1020145	1.00E-53	DNA binding protein

64	LI:789808.1:2000FEB01	g288424	0	Human ZNF37A mRNA for zinc finger protein.
65	LI:792919.1:2000FEB01	g2232012	0	Human zinc finger protein (FDZF2) mRNA, complete cds.
66	LI:793949.1:2000FEB01	g1017721	3.00E-53	Human repressor transcriptional factor (ZNF85) mRNA, complete cds.
67	LI:794389.1:2000FEB01	g5640017	4.00E-45	zinc finger protein ZFP113
68	LI:796010.1:2000FEB01	g288424	0	Human ZNF37A mRNA for zinc finger protein.
69	LI:796324.1:2000FEB01	g288424	0	Human ZNF37A mRNA for zinc finger protein.
70	LI:796373.1:2000FEB01	g1020145	9.00E-36	DNA binding protein
71	LI:796415.1:2000FEB01	g498151	4.00E-28	Human mRNA for KIAA0065 gene, partial cds.
72	LI:798636.1:2000FEB01	g2970037	0	Human HKL1 mRNA, complete cds.
73	LI:800045.1:2000FEB01	g538413	2.00E-55	zinc finger protein
74	LI:800680.1:2000FEB01	g7023216	7.00E-18	unnamed protein product (Homo sapiens)
75	LI:800894.1:2000FEB01	g3342001	0	Human hematopoietic cell derived zinc finger protein mRNA, complete
76	LI:801015.1:2000FEB01	g487785	4.00E-16	zinc finger protein ZNF136 (Homo sapiens)
77	LI:801236.1:2000FEB01	g488555	4.00E-48	zinc finger protein ZNF135
78	LI:803335.1:2000FEB01	g498152	1.00E-20	ha0946 protein is Kruppel-related.
79	LI:803998.1:2000FEB01	g1017722	1.00E-53	repressor transcriptional factor
80	LI:478757.1:2000FEB01	g498151	9.00E-27	Human mRNA for KIAA0065 gene, partial cds.
81	LI:808532.1:2000FEB01	g2232012	0	Human zinc finger protein (FDZF2) mRNA, complete cds.
82	LI:443073.1:2000FEB01	g4567179	3.00E-33	BC37295_1 (Homo sapiens)
83	LI:479671.1:2000FEB01	g487784	3.00E-38	Human zinc finger protein ZNF136.
84	LI:810078.1:2000FEB01	g498718	0	Human HZF1 mRNA for zinc finger protein.
85	LI:810224.1:2000FEB01	g288424	0	Human ZNF37A mRNA for zinc finger protein.
86	LI:817052.2:2000FEB01	g1020145	1.00E-51	DNA binding protein
87	LG:892274.1:2000MAY19	g6650686	2.00E-95	Human Y-linked zinc finger protein (ZF) gene, complete cds.
88	LG:1080959.1:2000MAY19	g5262560	2.00E-40	hypothetical protein (Homo sapiens)
89	LG:1054900.1:2000MAY19	g5262560	3.00E-35	hypothetical protein (Homo sapiens)
90	LG:1077357.1:2000MAY19	g10047297	2.00E-23	KIAA1611 protein (Homo sapiens)
91	LG:1084051.1:2000MAY19	g5931821	8.00E-79	dJ228H13.3 (zinc finger protein) (Homo sapiens)
92	LG:1076853.1:2000MAY19	g506502	1.00E-141	NK10 (Mus musculus)
93	LG:481631.10:2000MAY19	g7023216	1.00E-142	unnamed protein product (Homo sapiens)
94	LG:1088431.2:2000MAY19	g7023216	7.00E-18	unnamed protein product (Homo sapiens)
95	LI:401619.10:2000MAY01	g7959865	1.00E-18	PRO2032 (Homo sapiens)
96	LI:1144007.1:2000MAY01	g5360097	0	putative kruppel-related zinc finger protein NY-REN-23 antigen (Homo sapiens)
97	LI:331074.1:2000MAY01	g2149792	0	Roaz (Rattus norvegicus)
98	LI:1170349.1:2000MAY01	g487787	1.00E-45	zinc finger protein ZNF140 (Homo sapiens)

99	LG:335097.1:2000FEB18	g7020440	6.00E-25	unnamed protein product (Homo sapiens) Human hereditary haemochromatosis region, histone 2A-like protein gene, hereditary haemochromatosis (HLA-H) gene, RoRet gene, and sodium phosphate transporter (NP73) gene, complete cds. Human hereditary haemochromatosis region, histone 2A-like protein gene, hereditary haemochromatosis (HLA-H) gene, RoRet gene, and sodium phosphate transporter (NP73) gene, complete cds. inwardly rectifying potassium channel Kir5.1 calcium channel alpha-2-delta-C subunit (Mus musculus) tetradotoxin-resistant voltage-gated sodium channel (Homo sapiens) beta-alanine-sensitive neuronal GABA transporter (Rattus norvegicus) CTL1 protein (Homo sapiens) ESTs AU058081(E30812), AU058365(E50679), AU030138(E50679) correspond to a region of the predicted gene.; Similar to Spinacia oleracea mRNA for proteasome 37kD subunit.(X96974) Rhesus monkey cyclophilin A mRNA, complete cds. Similarity to B.subtilis DNAJ protein (SW:DNAJ_BACSU); cDNA EST yk437a1.5 comes from this gene Method: conceptual translation supplied by author; putative hybrid protein similar to HERV-H protease and HERV-E integrase (human endogenous retrovirus) testis specific DNAJ-homolog dnaJ protein (Thermotoga maritima) 25 kDa trypsin inhibitor (Homo sapiens) putative chaperonin (Arabidopsis thaliana) similar to Homo sapiens mRNA for KIAA0723 protein with GenBank Accession Number AB018266.1 protease PC6 isoform A (Homo sapiens) lymphocyte specific helicase ORF derived from D1 leader region and integrase coding region (Homo sapiens) Human mariner1 transposase gene, complete consensus sequence. ORF derived from D1 leader region and integrase coding region (Homo sapiens) similar to mitochondrial RNA splicing MSR4 like protein; cDNA EST EMBL:C09217 comes from this gene
100	LG:1076451.1:2000FEB18	g2088550	0	
101	LI:805478.1:2000FEB01	g2088550	0	
102	LG:101269.1:2000MAY19	g3953533	2.10E-56	
103	LI:331087.1:2000MAY01	g4186073	3.00E-41	
104	LI:410188.1:2000MAY01	g4838145	0	
105	LI:1188288.1:2000MAY01	g204220	0	
106	LI:427997.4:2000MAY01	g6996442	1.00E-48	
107	LG:451682.1:2000FEB18	g5091520	3.00E-75	
108	LG:1077283.1:2000FEB18	g2565302	0	
109	LG:481436.5:2000FEB18	g3873707	2.60E-34	
110	LI:793701.1:2000FEB01	g1049231	4.00E-33	
111	LI:373637.1:2000FEB01	g2286123	3.00E-50	
112	LG:239368.2:2000MAY19	g4981382	1.00E-11	
113	LI:053826.1:2000MAY01	g2943716	4.00E-67	
114	LI:449393.1:2000MAY01	g6957716	1.00E-128	
115	LI:1071427.96:2000MAY01	g9956070	1.00E-144	
116	LI:336338.8:2000MAY01	g9296929	2.00E-16	
117	LG:345527.1:2000FEB18	g805296	3.40E-176	
118	LG:1089383.1:2000FEB18	g2104910	3.00E-23	
119	LG:1092522.1:2000FEB18	g1263080	0	
120	LG:1093216.1:2000FEB18	g2104910	1.00E-23	
121	LI:270318.3:2000FEB01	g3880433	3.00E-12	

122	LI:335671.2:2000FEB01	g805296	1.00E-83	lymphocyte specific helicase
123	LI:793758.1:2000FEB01	g2104910	4.00E-26	ORF derived from D1 leader region and integrase coding region (Homo sapiens)
124	LI:803718.1:2000FEB01	g2104910	3.00E-23	ORF derived from D1 leader region and integrase coding region (Homo sapiens)
125	LI:412179.1:2000FEB01	g1263080	4.00E-93	Human mariner1 transposase gene, complete consensus sequence.
126	LI:815678.1:2000FEB01	g7020440	3.00E-12	unnamed protein product (Homo sapiens)
127	LI:481361.3:2000FEB01	g3776011	5.00E-25	RNA helicase
128	LG:247388.1:2000MAY19	g6016932	3.00E-127	dj620E11.1a (novel Helicase C-terminal domain and SNF2 N-terminal domains containing protein, similar to KIAA0308)
129	LG:255789.10:2000MAY19	g37542	2.00E-57	Human mRNA for U1 small nuclear RNP-specific C protein.
130	LI:787618.1:2000MAY01	g7020440	3.00E-12	unnamed protein product (Homo sapiens)
131	LI:331610.2:2000MAY01	g2599502	0	protocadherin 68 (Homo sapiens)
132	LG:982697.1:2000FEB18	g10436424	1.00E-25	unnamed protein product (Homo sapiens)
133	LG:1080896.1:2000FEB18	g5926696	0	Human genomic DNA, chromosome 6p21.3, HLA Class I region, section 8/20.
134	LI:811341.1:2000FEB01	g5926696	0	Human genomic DNA, chromosome 6p21.3, HLA Class I region, section 8/20.
135	LI:903225.1:2000FEB01	g5926710	0	Human genomic DNA, chromosome 6p21.3, HLA Class I region, section 20/20.
136	LI:242079.2:2000FEB01	g5926703	0	Human genomic DNA, chromosome 6p21.3, HLA Class I region, section 15/20.
137	LG:979580.1:2000MAY19	g9280152	7.00E-23	unnamed protein product (Macaca fascicularis)
138	LI:1169865.1:2000MAY01	g673417	1.00E-112	class II antigen (Homo sapiens)
139	LG:337818.2:2000FEB18	g404777	4.80E-84	cytochrome P-450 2B-Bx
140	LI:337818.1:2000FEB01	g203759	4.00E-58	cytochrome P-450(1)
141	LG:241577.4:2000MAY19	g2809498	1.50E-29	cytochrome c oxidase subunit IV
142	LG:344786.4:2000MAY19	g164981	2.00E-06	cytochrome P-450p-2 (Oryctolagus cuniculus)
143	LI:414307.1:2000FEB01	g30095	9.00E-48	collagen subunit (alpha-1 (X)) 3
144	LI:202943.2:2000FEB01	g391663	7.00E-06	hikaru genki type I product
145	LI:246194.2:2000FEB01	g1405821	1.00E-05	SULFATED SURFACE GLYCOPROTEIN 185
146	LI:815961.1:2000FEB01	g292045	0	Human mucin mRNA, partial cds.
147	LG:120744.1:2000MAY19	g4582324	1.00E-168	dJ708F5.1 (PUTATIVE novel Collagen alpha 1 LIKE protein) (Homo sapiens)
148	LI:757520.1:2000MAY01	g7161771	0	keratin (Homo sapiens)
149	LG:160570.1:2000FEB18	g466548	1.00E-46	NBL4
150	LI:350398.3:2000FEB01	g3724141	1.00E-06	myosin I

151	LI:221285.1:2000FEB01	g18218	2.00E-74	spoke protein
152	LI:401605.2:2000FEB01	g1755049	1.00E-15	myosin X
153	LI:329017.1:2000FEB01	g1813638	2.00E-51	PF20
154	LI:401322.1:2000FEB01	g38076	2.00E-30	Macaque mRNA for alpha-tubulin.
155	LG:403409.1:2000MAY19	g7303061	0	Khc-73 gene product (Drosophila melanogaster)
156	LG:233933.5:2000MAY19	g7385113	2.00E-18	ankyrin 1 (Bos taurus)
157	LI:290344.1:2000MAY01	g1353782	0	dystrophin-related protein 2 (Homo sapiens)
158	LI:410742.1:2000MAY01	g2290200	0	desmoglein 3 (Mus musculus)
159	LG:406568.1:2000MAY19	g28969	5.30E-44	64 Kd autoantigen
160	LI:283762.1:2000MAY01	g1469868	0	The KIAA0143 gene product is related to a putative C.elegans gene
161	LI:347687.1:2000MAY01	g387514	1.00E-123	encoded on cosmid C32D5. (Homo sapiens)
162	LI:1146510.1:2000MAY01	g2149291	3.00E-24	DM-20 protein (Mus musculus)
163	LG:451710.1:2000FEB18	g5816996	2.40E-42	defender against death 1 protein (Homo sapiens)
164	LG:455771.1:2000FEB18	g643074	1.70E-59	ribosomal protein L32-like protein
165	LG:452089.1:2000FEB18	g463252	1.90E-62	putative 40S ribosomal protein s12
166	LG:246415.1:2000FEB18	g296451	0	RL5 ribosomal protein
167	LG:414144.10:2000FEB18	g200785	1.80E-16	Human mRNA for ribosomal protein S26.
168	LG:1101445.1:2000FEB18	g1800114	0	ribosomal protein L7
169	LG:452134.1:2000FEB18	g550024	0	Human ribosomal protein L7 antisense mRNA gene, partial sequence.
170	LI:903021.1:2000FEB01	g36139	0	Human ribosomal protein S10 mRNA, complete cds.
171	LI:246422.1:2000FEB01	g409069	0	Human mRNA for ribosomal protein L7.
172	LG:449404.1:2000MAY19	g4886269	5.00E-66	Human mRNA for HBp 15/L22, complete cds.
173	LG:449413.1:2000MAY19	g643074	1.00E-70	putative ribosomal protein S14 (Arabidopsis thaliana)
174	LG:450105.1:2000MAY19	g643074	6.00E-76	putative 40S ribosomal protein s12 (Fragaria x ananassa)
175	LG:460809.1:2000MAY19	g36129	4.00E-54	putative 40S ribosomal protein s12 (Fragaria x ananassa)
176	LG:481781.1:2000MAY19	g2331301	1.00E-130	Human mRNA for ribosomal protein L31.
177	LG:1101153.1:2000MAY19	g2668748	2.00E-95	ribosomal protein S4 type I (Zea mays)
178	LI:257695.20:2000MAY01	g57714	1.00E-62	ribosomal protein L17 (Zea mays)
179	LI:455771.1:2000MAY01	g643074	6.00E-76	ribosomal protein S16 (AA 1-146) (Rattus rattus)
180	LI:274551.1:2000MAY01	g36145	2.00E-59	putative 40S ribosomal protein s12 (Fragaria x ananassa)
181	LI:035973.1:2000MAY01	g57121	8.00E-29	Human mRNA for ribosomal protein S12.
182	LG:978427.5:2000FEB18	g545998	2.50E-67	ribosomal protein L37 (Rattus norvegicus)
183	LG:247781.2:2000FEB18	g2352427	9.40E-29	tricarboxylate carrier (rats, liver, Peptide Mitochondrial Partial, 357 aa)
184	LI:034583.1:2000FEB01	g5815141	0	peroxisomal Ca-dependent solute carrier
				nuclear body associated kinase 1b

185	LI:333307.2:2000FEB01	g295671	0.0003	selected as a weak suppressor of a mutant of the subunit AC40 of DNA
186	LI:814710.2:2000FEB01	g178281	1.00E-46	dependant RNA polymerase I and III
187	LG:414732.1:2000MAY19	g183233	2.00E-54	AHNK nucleoprotein
188	LG:413910.6:2000MAY19	g7022046	1.00E-109	beta-glucuronidase precursor (EC 3.2.1.31)
189	LI:414732.2:2000MAY01	g183232	0	unnamed protein product (Homo sapiens)
190	LI:900264.2:2000MAY01	g414797	2.00E-81	Human beta-glucuronidase mRNA, complete cds.
191	LI:335593.1:2000MAY01	g3851553	5.00E-34	pyruvate dehydrogenase phosphatase (Bos taurus)
192	LI:1189543.1:2000MAY01	g7025507	0	RNA-binding protein Nova-2 (Homo sapiens)
193	LG:455450.1:2000FEB18	g4105111	2.10E-20	ventral neuron-specific protein 1 NOVA1 (Mus musculus)
194	LG:1040978.1:2000FEB18	g453189	3.30E-41	dehydrin 6
195	LG:446649.1:2000FEB18	g181960	2.00E-35	acyl carrier protein
196	LG:132147.3:2000FEB18	g6446606	0	Human endozepine (putative ligand of benzodiazepine receptor)
197	LI:036034.1:2000FEB01	g9622856	1.00E-33	mRNA, complete cds.
198	LG:162161.1:2000MAY19	g5823961	3.00E-87	E3 ubiquitin ligase SMURF1 (Homo sapiens)
199	LG:407214.10:2000MAY19	g9963839	4.00E-54	sorting nexin 15A (Homo sapiens)
200	LG:204626.1:2000MAY19	g3243240	5.10E-41	cl20B11.1 (ortholog of rat RSEC5 (mammalian exocyst complex subunit))
201	LI:007401.1:2000MAY01	g4512103	3.00E-81	(Homo sapiens)
202	LI:476342.1:2000MAY01	g790641	2.00E-21	lipase (Homo sapiens)
203	LI:1072759.1:2000MAY01	g2367625	4.00E-21	syntaxin 11
204	LG:998857.1:2000FEB18	g2731641	6.10E-13	rab11 binding protein (Bos taurus)
205	LG:482261.1:2000FEB18	g4003386	0	gamma-thionin (Hordeum vulgare)
206	LG:480328.1:2000FEB18	g246482	0	protein synthesis elongation factor 1-alpha (Rhodotorula mucilaginosa)
207	LG:311197.1:2000MAY19	g505033	8.00E-62	Fas-ligand associated factor 3
208	LG:1054883.1:2000MAY19	g325464	0	Human genomic DNA of 8p21.3-p22 anti-oncogene of hepatocellular
209	LG:399395.1:2000MAY19	g1177607	8.00E-10	colorectal and non-small cell lung cancer, segment 9/11.
210	LG:380497.2:2000MAY19	g10504238	3.00E-88	prohibitin (Human, mRNA, 1043 nt).
211	LI:272913.22:2000MAY01	g4982485	3.00E-59	mitogen inducible gene mig-2 (Homo sapiens)
				Human endogenous retrovirus type C oncovirus sequence.
				pva1 (Plasmodium vivax)
				hepatocellular carcinoma-related putative tumor suppressor (Homo
				apoptosis related protein APR-3 (Homo sapiens)

TABLE 2

SEQ ID NO:	Template ID	Start	Stop	Frame	Pfam Hit	Pfam Description	E-value
1	LG:1040582.1:2000FEB18	267	539	forward 3	aldo_ket_red	Aldo/keto reductase family	2.50E-51
2	LG:453570.1:2000FEB18	186	605	forward 3	Glyoxalase	Glyoxalase	3.80E-72
3	LG:408751.3:2000FEB18	194	1345	forward 2	Dihydroorotase	Dihydroorotase-like	1.40E-19
4	LI:090574.1:2000FEB01	60	776	forward 3	carb_anhydase	Eukaryotic-type carbonic anhydrase	9.70E-144
6	LI:332176.1:2000FEB01	2	961	forward 2	Glyco_hydro_31	Glycosyl hydrolases family 31	4.10E-144
7	LI:403248.2:2000FEB01	191	367	forward 2	Om_DAP_Arg_deC	Pyridoxal-dependent decarboxylase	1.40E-12
8	LG:220992.1:2000MAY19	156	1556	forward 3	Amidase	Amidase	1.10E-153
9	LG:1094571.1:2000MAY19	328	720	forward 1	FAD_Synth	Riboflavin kinase / FAD synthetase	2.30E-42
10	LI:350754.4:2000MAY01	855	1121	forward 3	Transglut_core	Transglutaminase-like superfamily	2.90E-47
10	LI:350754.4:2000MAY01	1455	2132	forward 3	Transglutamin_N	Transglutaminase family	3.20E-106
10	LI:350754.4:2000MAY01	54	413	forward 3	Transglutamin_N	Transglutaminase family	2.50E-63
11	LI:255828.29:2000MAY01	2	367	forward 2	PK	Pyruvate kinase	7.00E-71
11	LI:255828.29:2000MAY01	348	512	forward 3	PK	Pyruvate kinase	5.70E-24
12	LI:1190263.1:2000MAY01	281	1750	forward 2	Sulfatase	Sulfatase	8.60E-66
14	LG:999414.3:2000FEB18	718	1038	forward 1	7tm_1	7 transmembrane receptor (rhodopsin family)	3.60E-13
14	LG:999414.3:2000FEB18	1115	1453	forward 2	7tm_1	7 transmembrane receptor (rhodopsin family)	4.30E-07
18	LG:068682.1:2000FEB18	176	883	forward 2	pkinaase	Eukaryotic protein kinase domain	1.70E-65
19	LG:242665.1:2000FEB18	190	747	forward 1	ras	Ras family	2.30E-34
20	LG:241743.1:2000FEB18	199	345	forward 1	PH	PH domain	8.00E-06
22	LG:344886.1:2000MAY19	379	957	forward 1	ras	Ras family	1.70E-17
25	LG:898771.1:2000MAY19	525	662	forward 3	Fork_head	Fork head domain	1.70E-25
28	LI:1085273.2:2000MAY01	285	1070	forward 3	DSPc	Dual specificity phosphatase, catalytic domain	1.30E-39
29	LI:333138.2:2000MAY01	291	1016	forward 3	pkinaase	Eukaryotic protein kinase domain	2.00E-90
32	LG:998283.7:2000FEB18	370	630	forward 1	bromodomain	Bromodomain	2.60E-29
32	LG:998283.7:2000FEB18	4	153	forward 1	PHD	PHD-finger	1.90E-12
34	LI:175223.1:2000FEB01	210	431	forward 3	CSD	'Cold-shock' DNA-binding domain	1.40E-18
38	LG:021371.3:2000FEB18	932	1000	forward 2	zf-C2H2	Zinc finger, C2H2 type	2.10E-04
39	LG:475404.1:2000FEB18	176	328	forward 2	KRAB	KRAB box	1.10E-15
40	LG:979406.2:2000FEB18	85	273	forward 1	KRAB	KRAB box	2.40E-34
41	LG:410726.1:2000FEB18	646	834	forward 1	KRAB	KRAB box	2.10E-17
41	LG:410726.1:2000FEB18	274	558	forward 1	SCAN	SCAN domain	8.90E-55
43	LG:1076828.1:2000FEB18	448	516	forward 1	zf-C2H2	Zinc finger, C2H2 type	2.00E-07
44	LG:1076931.1:2000FEB18	173	310	forward 2	KRAB	KRAB box	3.40E-21
45	LG:1078121.1:2000FEB18	186	374	forward 3	KRAB	KRAB box	2.80E-41

46	LG:1079203.1:2000FEB18	421	489	forward 1 zf-C2H2	Zinc finger, C2H2 type	6.00E-06
46	LG:1079203.1:2000FEB18	647	715	forward 2 zf-C2H2	Zinc finger, C2H2 type	9.20E-05
47	LG:1082586.1:2000FEB18	414	536	forward 3 KRAB	KRAB box	6.80E-12
48	LG:1082774.1:2000FEB18	138	326	forward 3 KRAB	KRAB box	1.30E-40
49	LG:1082775.1:2000FEB18	45	230	forward 3 KRAB	KRAB box	7.10E-39
49	LG:1082775.1:2000FEB18	840	908	forward 3 zf-C2H2	Zinc finger, C2H2 type	4.40E-05
50	LG:1083120.1:2000FEB18	117	266	forward 3 KRAB	KRAB box	5.10E-22
51	LG:1087707.1:2000FEB18	162	350	forward 3 KRAB	KRAB box	2.80E-40
52	LG:1090915.1:2000FEB18	129	251	forward 3 KRAB	KRAB box	7.40E-22
53	LG:1094230.1:2000FEB18	120	308	forward 3 KRAB	KRAB box	3.70E-41
54	LG:474848.3:2000FEB18	253	441	forward 1 KRAB	KRAB box	2.10E-38
55	LI:251656.1:2000FEB01	242	310	forward 2 zf-C2H2	Zinc finger, C2H2 type	3.90E-08
56	LI:021371.1:2000FEB01	717	785	forward 3 zf-C2H2	Zinc finger, C2H2 type	2.10E-04
57	LI:133095.1:2000FEB01	539	607	forward 2 zf-C2H2	Zinc finger, C2H2 type	4.30E-06
58	LI:236654.2:2000FEB01	805	873	forward 1 zf-C2H2	Zinc finger, C2H2 type	1.40E-04
59	LI:200009.1:2000FEB01	564	632	forward 3 zf-C2H2	Zinc finger, C2H2 type	1.80E-05
60	LI:758502.1:2000FEB01	633	701	forward 3 zf-C2H2	Zinc finger, C2H2 type	2.50E-07
62	LI:789445.1:2000FEB01	71	262	forward 2 KRAB	KRAB box	1.60E-27
63	LI:789657.1:2000FEB01	542	610	forward 2 zf-C2H2	Zinc finger, C2H2 type	2.60E-06
64	LI:789808.1:2000FEB01	272	340	forward 2 zf-C2H2	Zinc finger, C2H2 type	1.00E-07
64	LI:789808.1:2000FEB01	426	494	forward 3 zf-C2H2	Zinc finger, C2H2 type	3.40E-04
65	LI:792919.1:2000FEB01	31	99	forward 1 zf-C2H2	Zinc finger, C2H2 type	5.30E-06
66	LI:793949.1:2000FEB01	120	308	forward 3 KRAB	KRAB box	1.70E-41
67	LI:794389.1:2000FEB01	75	143	forward 3 zf-C2H2	Zinc finger, C2H2 type	8.70E-06
68	LI:796010.1:2000FEB01	276	344	forward 3 zf-C2H2	Zinc finger, C2H2 type	1.00E-07
68	LI:796010.1:2000FEB01	433	501	forward 1 zf-C2H2	Zinc finger, C2H2 type	3.40E-04
69	LI:796324.1:2000FEB01	290	358	forward 2 zf-C2H2	Zinc finger, C2H2 type	1.00E-07
69	LI:796324.1:2000FEB01	450	518	forward 3 zf-C2H2	Zinc finger, C2H2 type	3.40E-04
70	LI:796373.1:2000FEB01	181	249	forward 1 zf-C2H2	Zinc finger, C2H2 type	1.10E-06
71	LI:796415.1:2000FEB01	45	230	forward 3 KRAB	KRAB box	7.10E-39
72	LI:798636.1:2000FEB01	329	397	forward 2 zf-C2H2	Zinc finger, C2H2 type	2.60E-07
73	LI:800045.1:2000FEB01	364	432	forward 1 zf-C2H2	Zinc finger, C2H2 type	5.30E-07
74	LI:800680.1:2000FEB01	155	319	forward 2 KRAB	KRAB box	5.00E-21
75	LI:800894.1:2000FEB01	125	313	forward 2 KRAB	KRAB box	6.50E-40
76	LI:801015.1:2000FEB01	22	216	forward 1 KRAB	KRAB box	3.00E-24
77	LI:801236.1:2000FEB01	225	293	forward 3 zf-C2H2	Zinc finger, C2H2 type	4.40E-07

78	LI:803335.1:2000FEB01	220	408	forward 1 KRAB	KRAB box	2.10E-38
79	LI:803998.1:2000FEB01	62	130	forward 2 zf-C2H2	Zinc finger, C2H2 type	1.20E-05
80	LI:478757.1:2000FEB01	467	643	forward 2 KRAB	KRAB box	2.40E-21
81	LI:808532.1:2000FEB01	53	121	forward 2 zf-C2H2	Zinc finger, C2H2 type	5.70E-05
82	LI:443073.1:2000FEB01	176	244	forward 2 zf-C2H2	Zinc finger, C2H2 type	2.50E-05
83	LI:479671.1:2000FEB01	160	312	forward 1 KRAB	KRAB box	1.70E-19
84	LI:810078.1:2000FEB01	424	492	forward 1 zf-C2H2	Zinc finger, C2H2 type	1.80E-06
84	LI:810078.1:2000FEB01	587	655	forward 2 zf-C2H2	Zinc finger, C2H2 type	1.20E-05
85	LI:810224.1:2000FEB01	171	239	forward 3 zf-C2H2	Zinc finger, C2H2 type	1.00E-07
86	LI:817052.2:2000FEB01	901	969	forward 1 zf-C2H2	Zinc finger, C2H2 type	8.90E-08
87	LG:892274.1:2000MAY19	96	461	forward 3 dUTPase	dUTPase	9.20E-27
87	LG:892274.1:2000MAY19	489	752	forward 3 rvp	Retroviral aspartyl protease	5.30E-11
88	LG:1080959.1:2000MAY19	182	322	forward 2 KRAB	KRAB box	2.00E-16
89	LG:1054900.1:2000MAY19	78	218	forward 3 KRAB	KRAB box	2.30E-17
90	LG:1077357.1:2000MAY19	94	282	forward 1 KRAB	KRAB box	4.80E-31
91	LG:1084051.1:2000MAY19	195	263	forward 3 zf-C2H2	Zinc finger, C2H2 type	1.80E-06
92	LG:1076853.1:2000MAY19	706	774	forward 1 zf-C2H2	Zinc finger, C2H2 type	1.50E-07
93	LG:481631.10:2000MAY19	96	263	forward 3 KRAB	KRAB box	5.70E-25
93	LG:481631.10:2000MAY19	882	950	forward 3 zf-C2H2	Zinc finger, C2H2 type	1.70E-05
94	LG:1088431.2:2000MAY19	175	339	forward 1 KRAB	KRAB box	5.00E-21
96	LI:1144007.1:2000MAY01	914	1108	forward 2 KRAB	KRAB box	5.90E-05
96	LI:1144007.1:2000MAY01	323	610	forward 2 SCAN	SCAN domain	4.10E-60
97	LI:331074.1:2000MAY01	194	262	forward 2 zf-C2H2	Zinc finger, C2H2 type	1.00E-03
98	LI:1170349.1:2000MAY01	185	370	forward 2 KRAB	KRAB box	2.50E-29
98	LI:1170349.1:2000MAY01	740	808	forward 2 zf-C2H2	Zinc finger, C2H2 type	5.80E-05
102	LG:101269.1:2000MAY19	556	831	forward 1 IRK	Inward rectifier potassium channel	3.50E-65
104	LI:410188.1:2000MAY01	3760	4569	forward 1 ion_trans	Ion transport protein	3.70E-97
104	LI:410188.1:2000MAY01	4586	5314	forward 2 ion_trans	Ion transport protein	3.30E-66
105	LI:1188288.1:2000MAY01	751	1215	forward 1 SNF	Sodium:neurotransmitter symporter family	8.50E-113
105	LI:1188288.1:2000MAY01	423	782	forward 3 SNF	Sodium:neurotransmitter symporter family	8.60E-74
105	LI:1188288.1:2000MAY01	1187	1438	forward 2 SNF	Sodium:neurotransmitter symporter family	5.50E-52
107	LG:451682.1:2000FEB18	117	560	forward 3 proteasome	Proteasome A-type and B-type	4.40E-59
108	LG:1077283.1:2000FEB18	110	427	forward 2 pro_isomerase	Cyclophilin type peptidyl-prolyl cis-trans isomerase	1.80E-37
108	LG:1077283.1:2000FEB18	177	278	forward 3 pro_isomerase	Cyclophilin type peptidyl-prolyl cis-trans isomerase	1.30E-18
109	LG:481436.5:2000FEB18	351	539	forward 3 DnaJ	DnaJ domain	2.80E-28
111	LI:373637.1:2000FEB01	17	217	forward 2 DnaJ	DnaJ domain	6.30E-39

113	LI:053826.1:2000MAY01	834	1106	forward 3 SCP	SCP-like extracellular protein	1.10E-17
114	LI:449393.1:2000MAY01	90	788	forward 3 cnp60_TCP1	TCP-1/cnp60 chaperonin family	9.80E-66
117	LG:345527.1:2000FEB18	667	957	forward 1 helicase_C	Helicases conserved C-terminal domain	7.20E-21
117	LG:345527.1:2000FEB18	8	631	forward 2 SNF2_N	SNF2 and others N-terminal domain	7.20E-44
122	LI:335671.2:2000FEB01	188	475	forward 2 helicase_C	Helicases conserved C-terminal domain	9.10E-13
122	LI:335671.2:2000FEB01	3	95	forward 3 SNF2_N	SNF2 and others N-terminal domain	7.10E-06
128	LG:247388.1:2000MAY19	346	600	forward 1 helicase_C	Helicases conserved C-terminal domain	2.70E-19
128	LG:247388.1:2000MAY19	3	173	forward 3 SNF2_N	SNF2 and others N-terminal domain	1.60E-14
131	LI:331610.2:2000MAY01	1415	1699	forward 2 cadherin	Cadherin domain	6.00E-20
135	LI:903225.1:2000FEB01	603	764	forward 3 Ribosomal_L23	Ribosomal protein L23	4.80E-14
138	LI:1169865.1:2000MAY01	593	790	forward 2 ig	Immunoglobulin domain	2.30E-08
138	LI:1169865.1:2000MAY01	242	547	forward 2 MHC_IL_alpha	Class II histocompatibility antigen, alpha domain	1.80E-65
139	LG:337818.2:2000FEB18	136	1518	forward 1 p450	Cytochrome P450	1.50E-173
140	LI:337818.1:2000FEB01	654	998	forward 3 p450	Cytochrome P450	3.50E-45
140	LI:337818.1:2000FEB01	136	384	forward 1 p450	Cytochrome P450	4.40E-27
140	LI:337818.1:2000FEB01	359	673	forward 2 p450	Cytochrome P450	5.40E-27
143	LI:414307.1:2000FEB01	590	964	forward 2 C1q	C1q domain	2.30E-38
143	LI:414307.1:2000FEB01	365	544	forward 2 Collagen	Collagen triple helix repeat (20 copies)	2.50E-10
144	LI:202943.2:2000FEB01	36	209	forward 3 sushi	Sushi domain (SCR repeat)	1.40E-09
147	LG:120744.1:2000MAY19	301	813	forward 1 vwa	von Willebrand factor type A domain	2.00E-51
148	LI:757520.1:2000MAY01	427	1362	forward 1 filament	Intermediate filament proteins	7.10E-157
149	LG:160570.1:2000FEB18	260	562	forward 2 Band_41	FERM domain (Band 4.1 family)	1.60E-22
152	LI:401605.2:2000FEB01	1	129	forward 1 myosin_head	Myosin head (motor domain)	5.90E-07
153	LI:329017.1:2000FEB01	226	336	forward 1 WD40	WD domain, G-beta repeat	5.10E-06
154	LI:401322.1:2000FEB01	156	341	forward 3 tubulin	Tubulin/FtsZ family	7.10E-20
154	LI:401322.1:2000FEB01	371	478	forward 2 tubulin	Tubulin/FtsZ family	2.50E-06
155	LG:403409.1:2000MAY19	1458	1652	forward 3 FHA	FHA domain	3.00E-04
155	LG:403409.1:2000MAY19	78	1193	forward 3 kinesin	Kinesin motor domain	6.80E-172
156	LG:233933.5:2000MAY19	258	356	forward 3 ank	Ank repeat	4.90E-06
157	LI:290344.1:2000MAY01	992	1312	forward 2 spectrin	Spectrin repeat	4.10E-07
157	LI:290344.1:2000MAY01	1361	1450	forward 2 VW	VW domain	5.40E-08
158	LI:410742.1:2000MAY01	599	889	forward 2 cadherin	Cadherin domain	1.80E-21
158	LI:410742.1:2000MAY01	1224	1520	forward 3 cadherin	Cadherin domain	9.90E-04
161	LI:347687.113:2000MAY01	214	855	forward 1 Myelin_PLP	Myelin proteolipid protein (PLP or lipophilin)	7.10E-160
163	LG:451710.1:2000FEB18	130	459	forward 1 Ribosomal_L32e	Ribosomal protein L32	4.80E-57
164	LG:455771.1:2000FEB18	69	473	forward 3 Ribosomal_S12	Ribosomal protein S12	6.60E-78

165	LG:452089.1:2000FEB18	107	268	forward 2 Ribosomal_L5	Ribosomal protein L5	2.40E-25
165	LG:452089.1:2000FEB18	278	577	forward 2 Ribosomal_L5_C	ribosomal L5P family C-terminus	2.70E-60
166	LG:246415.1:2000FEB18	27	365	forward 3 Ribosomal_S26e	Ribosomal protein S26e	2.40E-59
168	LG:1101445.1:2000FEB18	306	464	forward 3 Ribosomal_L30	Ribosomal protein L30p/L7e	4.20E-28
171	LI:246422.1:2000FEB01	53	397	forward 2 Ribosomal_L22e	Ribosomal L22e protein family	4.30E-28
171	LI:246422.1:2000FEB01	64	318	forward 1 Ribosomal_L22e	Ribosomal L22e protein family	7.70E-07
172	LG:449404.1:2000MAY19	175	531	forward 1 Ribosomal_S11	Ribosomal protein S11	6.90E-77
173	LG:449413.1:2000MAY19	99	368	forward 3 Ribosomal_S12	Ribosomal protein S12	6.10E-47
173	LG:449413.1:2000MAY19	367	504	forward 1 Ribosomal_S12	Ribosomal protein S12	3.20E-21
174	LG:450105.1:2000MAY19	86	490	forward 2 Ribosomal_S12	Ribosomal protein S12	6.60E-78
175	LG:460809.1:2000MAY19	3	236	forward 3 Ribosomal_L31e	Ribosomal protein L31e	6.00E-17
176	LG:481781.1:2000MAY19	243	671	forward 3 Ribosomal_S4e	Ribosomal family S4e	1.40E-97
177	LG:1101153.1:2000MAY19	89	499	forward 2 Ribosomal_L22	Ribosomal protein L22p/L17e	5.20E-76
178	LI:257695.20:2000MAY01	110	673	forward 2 Ribosomal_S9	Ribosomal protein S9/S16	1.60E-40
179	LI:455771.1:2000MAY01	69	473	forward 3 Ribosomal_S12	Ribosomal protein S12	6.60E-78
181	LI:035973.1:2000MAY01	318	479	forward 3 Ribosomal_L37e	Ribosomal protein L37e	1.60E-13
183	LG:247781.2:2000FEB18	142	426	forward 1 mito_carr	Mitochondrial carrier proteins	1.80E-24
190	LI:900264.2:2000MAY01	1151	1555	forward 2 PP2C	Protein phosphatase 2C	2.90E-11
192	LI:1189543.1:2000MAY01	1292	1447	forward 2 KH-domain	KH domain	2.50E-13
192	LI:1189543.1:2000MAY01	592	744	forward 1 KH-domain	KH domain	5.40E-13
193	LG:455450.1:2000FEB18	1	426	forward 1 dehydrin	Dehydrins	4.20E-41
194	LG:1040978.1:2000FEB18	278	481	forward 2 pp-binding	Phosphopantetheine attachment site	3.90E-14
195	LG:446649.1:2000FEB18	80	316	forward 2 ACBP	Acyl CoA binding protein	1.60E-44
196	LG:132147.3:2000FEB18	1497	2414	forward 3 HECT	HECT-domain (ubiquitin-transferase).	9.90E-138
196	LG:132147.3:2000FEB18	1065	1154	forward 3 WW	WW domain	1.50E-12
198	LG:162161.1:2000MAY19	128	385	forward 2 TIG	IP/TIG domain	5.50E-15
200	LG:204626.1:2000MAY19	322	1212	forward 1 Syntaxin	Syntaxin	8.60E-44
202	LI:476342.1:2000MAY01	159	299	forward 3 Gamma-thionin	Gamma-thionins family	1.70E-19
205	LG:482261.1:2000FEB18	286	552	forward 1 Gag_p10	Retroviral GAG p10 protein	6.80E-31
205	LG:482261.1:2000FEB18	1044	1229	forward 3 gag_p24	gag gene protein p24 (core nucleocapsid	2.00E-15
205	LG:482261.1:2000FEB18	1375	1545	forward 1 gag_p24	gag gene protein p24 (core nucleocapsid	9.50E-15
206	LG:480328.1:2000FEB18	985	1515	forward 1 Band_7	SPFH domain / Band 7 family	2.60E-39
206	LG:480328.1:2000FEB18	49	117	forward 1 zf-C2H2	Zinc finger, C2H2 type	1.60E-06
210	LG:380497.2:2000MAY19	202	336	forward 1 G-patch	G-patch domain	7.00E-17

TABLE 3						
Template ID		Start	Stop	Frame	Domain	Topology
SEQ ID NO:					Type	
1	LG:1040582.1:2000FEB18	31	117	forward 1	TM	N in
1	LG:1040582.1:2000FEB18	319	405	forward 1	TM	N in
1	LG:1040582.1:2000FEB18	108	155	forward 3	TM	N out
2	LG:453570.1:2000FEB18	361	447	forward 1	TM	N in
3	LG:408751.3:2000FEB18	1318	1404	forward 1	TM	N in
3	LG:408751.3:2000FEB18	1025	1099	forward 2	TM	N in
3	LG:408751.3:2000FEB18	1298	1360	forward 2	TM	N in
3	LG:408751.3:2000FEB18	1379	1441	forward 2	TM	N in
3	LG:408751.3:2000FEB18	1463	1537	forward 2	TM	N in
3	LG:408751.3:2000FEB18	1047	1133	forward 3	TM	N in
3	LG:408751.3:2000FEB18	1266	1352	forward 3	TM	N in
3	LG:408751.3:2000FEB18	1419	1469	forward 3	TM	N in
4	LI:090574.1:2000FEB01	79	144	forward 1	TM	N in
4	LI:090574.1:2000FEB01	607	678	forward 1	TM	N in
4	LI:090574.1:2000FEB01	1009	1080	forward 1	TM	N in
4	LI:090574.1:2000FEB01	497	583	forward 2	TM	N out
4	LI:090574.1:2000FEB01	743	829	forward 2	TM	N out
4	LI:090574.1:2000FEB01	1026	1085	forward 3	TM	N out
5	LI:229932.2:2000FEB01	76	162	forward 1	TM	N out
5	LI:229932.2:2000FEB01	190	276	forward 1	TM	N out
5	LI:229932.2:2000FEB01	1237	1323	forward 1	TM	N out
5	LI:229932.2:2000FEB01	68	142	forward 2	TM	N in
5	LI:229932.2:2000FEB01	335	412	forward 2	TM	N in
5	LI:229932.2:2000FEB01	758	844	forward 2	TM	N in
5	LI:229932.2:2000FEB01	1229	1288	forward 2	TM	N in
5	LI:229932.2:2000FEB01	60	146	forward 3	TM	N in
5	LI:229932.2:2000FEB01	216	302	forward 3	TM	N in
5	LI:229932.2:2000FEB01	690	752	forward 3	TM	N in
5	LI:229932.2:2000FEB01	765	827	forward 3	TM	N in
5	LI:229932.2:2000FEB01	1209	1289	forward 3	TM	N in
6	LI:332176.1:2000FEB01	343	399	forward 1	TM	N in
6	LI:332176.1:2000FEB01	1078	1131	forward 1	TM	N in
6	LI:332176.1:2000FEB01	1606	1692	forward 1	TM	N in
6	LI:332176.1:2000FEB01	2218	2274	forward 1	TM	N in
6	LI:332176.1:2000FEB01	2383	2433	forward 1	TM	N in
6	LI:332176.1:2000FEB01	110	196	forward 2	TM	N in
6	LI:332176.1:2000FEB01	1307	1378	forward 2	TM	N in
6	LI:332176.1:2000FEB01	1640	1726	forward 2	TM	N in
6	LI:332176.1:2000FEB01	1946	2005	forward 2	TM	N in
6	LI:332176.1:2000FEB01	135	200	forward 3	TM	N in
6	LI:332176.1:2000FEB01	693	752	forward 3	TM	N in
6	LI:332176.1:2000FEB01	777	839	forward 3	TM	N in
6	LI:332176.1:2000FEB01	867	929	forward 3	TM	N in
6	LI:332176.1:2000FEB01	1035	1118	forward 3	TM	N in
6	LI:332176.1:2000FEB01	1173	1253	forward 3	TM	N in
6	LI:332176.1:2000FEB01	1572	1658	forward 3	TM	N in
6	LI:332176.1:2000FEB01	2121	2180	forward 3	TM	N in
6	LI:332176.1:2000FEB01	2277	2363	forward 3	TM	N in
6	LI:332176.1:2000FEB01	2400	2456	forward 3	TM	N in
8	LG:220992.1:2000MAY19	343	393	forward 1	TM	
8	LG:220992.1:2000MAY19	646	732	forward 1	TM	
8	LG:220992.1:2000MAY19	1639	1725	forward 1	TM	
8	LG:220992.1:2000MAY19	1879	1965	forward 1	TM	

8	LG:220992.1:2000MAY19	2005	2088	forward 1	TM	
8	LG:220992.1:2000MAY19	17	76	forward 2	TM	N in
8	LG:220992.1:2000MAY19	1646	1732	forward 2	TM	N in
8	LG:220992.1:2000MAY19	1850	1933	forward 2	TM	N in
8	LG:220992.1:2000MAY19	1434	1484	forward 3	TM	N out
8	LG:220992.1:2000MAY19	1734	1820	forward 3	TM	N out
8	LG:220992.1:2000MAY19	1974	2036	forward 3	TM	N out
8	LG:220992.1:2000MAY19	2067	2129	forward 3	TM	N out
8	LG:220992.1:2000MAY19	2151	2237	forward 3	TM	N out
9	LG:1094571.1:2000MAY19	781	867	forward 1	TM	N in
9	LG:1094571.1:2000MAY19	419	505	forward 2	TM	N in
9	LG:1094571.1:2000MAY19	767	853	forward 2	TM	N in
9	LG:1094571.1:2000MAY19	756	842	forward 3	TM	N in
10	LI:350754.4:2000MAY01	277	348	forward 1	TM	N in
10	LI:350754.4:2000MAY01	583	651	forward 1	TM	N in
10	LI:350754.4:2000MAY01	670	747	forward 1	TM	N in
10	LI:350754.4:2000MAY01	381	467	forward 3	TM	N in
10	LI:350754.4:2000MAY01	2469	2555	forward 3	TM	N in
12	LI:1190263.1:2000MAY01	664	735	forward 1	TM	N in
12	LI:1190263.1:2000MAY01	787	861	forward 1	TM	N in
12	LI:1190263.1:2000MAY01	901	954	forward 1	TM	N in
12	LI:1190263.1:2000MAY01	188	274	forward 2	TM	N in
12	LI:1190263.1:2000MAY01	455	508	forward 2	TM	N in
12	LI:1190263.1:2000MAY01	809	895	forward 2	TM	N in
12	LI:1190263.1:2000MAY01	1616	1663	forward 2	TM	N in
12	LI:1190263.1:2000MAY01	183	251	forward 3	TM	N in
12	LI:1190263.1:2000MAY01	648	704	forward 3	TM	N in
12	LI:1190263.1:2000MAY01	1149	1235	forward 3	TM	N in
13	LG:270916.2:2000FEB18	173	259	forward 2	TM	N out
14	LG:999414.3:2000FEB18	109	195	forward 1	TM	N out
14	LG:999414.3:2000FEB18	358	438	forward 1	TM	N out
14	LG:999414.3:2000FEB18	520	591	forward 1	TM	N out
14	LG:999414.3:2000FEB18	661	744	forward 1	TM	N out
14	LG:999414.3:2000FEB18	883	969	forward 1	TM	N out
14	LG:999414.3:2000FEB18	976	1062	forward 1	TM	N out
14	LG:999414.3:2000FEB18	302	388	forward 2	TM	N in
14	LG:999414.3:2000FEB18	533	613	forward 2	TM	N in
14	LG:999414.3:2000FEB18	992	1048	forward 2	TM	N in
14	LG:999414.3:2000FEB18	1169	1246	forward 2	TM	N in
14	LG:999414.3:2000FEB18	1307	1366	forward 2	TM	N in
14	LG:999414.3:2000FEB18	207	284	forward 3	TM	N out
14	LG:999414.3:2000FEB18	324	404	forward 3	TM	N out
14	LG:999414.3:2000FEB18	540	599	forward 3	TM	N out
14	LG:999414.3:2000FEB18	1029	1115	forward 3	TM	N out
14	LG:999414.3:2000FEB18	1167	1253	forward 3	TM	N out
14	LG:999414.3:2000FEB18	1314	1373	forward 3	TM	N out
15	LG:429446.1:2000FEB18	628	699	forward 1	TM	N out
15	LG:429446.1:2000FEB18	629	682	forward 2	TM	N in
15	LG:429446.1:2000FEB18	627	713	forward 3	TM	N in
16	LI:057229.1:2000FEB01	10	69	forward 1	TM	
16	LI:057229.1:2000FEB01	118	198	forward 1	TM	
16	LI:057229.1:2000FEB01	292	360	forward 1	TM	
16	LI:057229.1:2000FEB01	11	67	forward 2	TM	
16	LI:057229.1:2000FEB01	146	226	forward 2	TM	
16	LI:057229.1:2000FEB01	290	355	forward 2	TM	
16	LI:057229.1:2000FEB01	12	71	forward 3	TM	N out

16	LI:057229.1:2000FEB01	114	176	forward 3	TM	N out
17	LI:351965.1:2000FEB01	487	573	forward 1	TM	
17	LI:351965.1:2000FEB01	1036	1098	forward 1	TM	
17	LI:351965.1:2000FEB01	492	578	forward 3	TM	N in
17	LI:351965.1:2000FEB01	969	1055	forward 3	TM	N in
17	LI:351965.1:2000FEB01	1098	1184	forward 3	TM	N in
18	LG:068682.1:2000FEB18	707	793	forward 2	TM	N out
19	LG:242665.1:2000FEB18	10	63	forward 1	TM	N out
19	LG:242665.1:2000FEB18	12	62	forward 3	TM	N out
19	LG:242665.1:2000FEB18	333	398	forward 3	TM	N out
20	LG:241743.1:2000FEB18	43	99	forward 1	TM	N out
21	LI:034212.1:2000FEB01	1300	1365	forward 1	TM	N in
21	LI:034212.1:2000FEB01	1570	1647	forward 1	TM	N in
21	LI:034212.1:2000FEB01	2386	2472	forward 1	TM	N in
21	LI:034212.1:2000FEB01	2533	2598	forward 1	TM	N in
21	LI:034212.1:2000FEB01	2620	2706	forward 1	TM	N in
21	LI:034212.1:2000FEB01	2740	2826	forward 1	TM	N in
21	LI:034212.1:2000FEB01	719	805	forward 2	TM	
21	LI:034212.1:2000FEB01	1205	1291	forward 2	TM	
21	LI:034212.1:2000FEB01	1460	1546	forward 2	TM	
21	LI:034212.1:2000FEB01	1685	1768	forward 2	TM	
21	LI:034212.1:2000FEB01	1814	1882	forward 2	TM	
21	LI:034212.1:2000FEB01	2066	2128	forward 2	TM	
21	LI:034212.1:2000FEB01	2156	2218	forward 2	TM	
21	LI:034212.1:2000FEB01	2540	2626	forward 2	TM	
21	LI:034212.1:2000FEB01	2657	2734	forward 2	TM	
21	LI:034212.1:2000FEB01	12	62	forward 3	TM	N out
21	LI:034212.1:2000FEB01	1236	1301	forward 3	TM	N out
21	LI:034212.1:2000FEB01	1590	1646	forward 3	TM	N out
21	LI:034212.1:2000FEB01	1668	1721	forward 3	TM	N out
21	LI:034212.1:2000FEB01	2130	2216	forward 3	TM	N out
21	LI:034212.1:2000FEB01	2295	2381	forward 3	TM	N out
21	LI:034212.1:2000FEB01	2436	2513	forward 3	TM	N out
21	LI:034212.1:2000FEB01	2538	2624	forward 3	TM	N out
21	LI:034212.1:2000FEB01	2667	2735	forward 3	TM	N out
22	LG:344886.1:2000MAY19	937	1002	forward 1	TM	N in
22	LG:344886.1:2000MAY19	1081	1155	forward 1	TM	N in
22	LG:344886.1:2000MAY19	1696	1782	forward 1	TM	N in
22	LG:344886.1:2000MAY19	413	463	forward 2	TM	N in
22	LG:344886.1:2000MAY19	551	637	forward 2	TM	N in
22	LG:344886.1:2000MAY19	950	1012	forward 2	TM	N in
22	LG:344886.1:2000MAY19	1031	1093	forward 2	TM	N in
22	LG:344886.1:2000MAY19	1112	1183	forward 2	TM	N in
22	LG:344886.1:2000MAY19	1271	1348	forward 2	TM	N in
22	LG:344886.1:2000MAY19	1634	1720	forward 2	TM	N in
22	LG:344886.1:2000MAY19	567	626	forward 3	TM	N in
22	LG:344886.1:2000MAY19	1011	1073	forward 3	TM	N in
22	LG:344886.1:2000MAY19	1089	1151	forward 3	TM	N in
22	LG:344886.1:2000MAY19	1707	1757	forward 3	TM	N in
23	LG:228930.1:2000MAY19	111	167	forward 3	TM	N in
24	LG:338927.1:2000MAY19	934	1020	forward 1	TM	N out
24	LG:338927.1:2000MAY19	1133	1219	forward 2	TM	N in
24	LG:338927.1:2000MAY19	1170	1250	forward 3	TM	N in
25	LG:898771.1:2000MAY19	1261	1314	forward 1	TM	N out
25	LG:898771.1:2000MAY19	1397	1450	forward 2	TM	N out
26	LI:257664.67:2000MAY01	280	366	forward 1	TM	N in

26	LI:257664.67:2000MAY01	421	498	forward 1	TM	N in
26	LI:257664.67:2000MAY01	12	71	forward 3	TM	N out
27	LI:001496.2:2000MAY01	399	473	forward 3	TM	
28	LI:1085273.2:2000MAY01	2188	2274	forward 1	TM	N in
28	LI:1085273.2:2000MAY01	503	583	forward 2	TM	N out
28	LI:1085273.2:2000MAY01	2126	2194	forward 2	TM	N out
28	LI:1085273.2:2000MAY01	897	968	forward 3	TM	N in
29	LI:333138.2:2000MAY01	1930	2016	forward 1	TM	N out
29	LI:333138.2:2000MAY01	50	103	forward 2	TM	
29	LI:333138.2:2000MAY01	884	940	forward 2	TM	
29	LI:333138.2:2000MAY01	114	179	forward 3	TM	N out
29	LI:333138.2:2000MAY01	273	356	forward 3	TM	N out
29	LI:333138.2:2000MAY01	819	875	forward 3	TM	N out
29	LI:333138.2:2000MAY01	1581	1667	forward 3	TM	N out
30	LI:338927.1:2000MAY01	1069	1140	forward 1	TM	N in
30	LI:338927.1:2000MAY01	968	1051	forward 2	TM	N in
30	LI:338927.1:2000MAY01	1056	1118	forward 3	TM	N out
30	LI:338927.1:2000MAY01	1155	1217	forward 3	TM	N out
31	LG:335558.1:2000FEB18	518	604	forward 2	TM	N in
31	LG:335558.1:2000FEB18	614	682	forward 2	TM	N in
31	LG:335558.1:2000FEB18	761	829	forward 2	TM	N in
31	LG:335558.1:2000FEB18	798	860	forward 3	TM	N in
31	LG:335558.1:2000FEB18	882	944	forward 3	TM	N in
31	LG:335558.1:2000FEB18	966	1028	forward 3	TM	N in
32	LG:998283.7:2000FEB18	1066	1146	forward 1	TM	N in
32	LG:998283.7:2000FEB18	23	109	forward 2	TM	N in
32	LG:998283.7:2000FEB18	194	280	forward 2	TM	N in
32	LG:998283.7:2000FEB18	392	478	forward 2	TM	N in
32	LG:998283.7:2000FEB18	527	613	forward 2	TM	N in
32	LG:998283.7:2000FEB18	776	862	forward 2	TM	N in
32	LG:998283.7:2000FEB18	1064	1141	forward 2	TM	N in
32	LG:998283.7:2000FEB18	12	65	forward 3	TM	N in
32	LG:998283.7:2000FEB18	147	227	forward 3	TM	N in
32	LG:998283.7:2000FEB18	684	770	forward 3	TM	N in
32	LG:998283.7:2000FEB18	1011	1097	forward 3	TM	N in
33	LI:402739.1:2000FEB01	415	501	forward 1	TM	N in
35	LG:981076.2:2000MAY19	388	450	forward 1	TM	N in
35	LG:981076.2:2000MAY19	20	82	forward 2	TM	N out
35	LG:981076.2:2000MAY19	389	451	forward 2	TM	N out
35	LG:981076.2:2000MAY19	464	526	forward 2	TM	N out
35	LG:981076.2:2000MAY19	539	604	forward 2	TM	N out
35	LG:981076.2:2000MAY19	438	524	forward 3	TM	N in
37	LI:1190250.1:2000MAY01	530	613	forward 2	TM	
37	LI:1190250.1:2000MAY01	558	635	forward 3	TM	N out
38	LG:021371.3:2000FEB18	122	208	forward 2	TM	N in
41	LG:410726.1:2000FEB18	22	108	forward 1	TM	N in
41	LG:410726.1:2000FEB18	385	471	forward 1	TM	N in
42	LG:200005.1:2000FEB18	166	222	forward 1	TM	N out
42	LG:200005.1:2000FEB18	185	232	forward 2	TM	N out
42	LG:200005.1:2000FEB18	162	248	forward 3	TM	N out
46	LG:1079203.1:2000FEB18	11	70	forward 2	TM	N in
46	LG:1079203.1:2000FEB18	125	196	forward 2	TM	N in
46	LG:1079203.1:2000FEB18	965	1051	forward 2	TM	N in
47	LG:1082586.1:2000FEB18	256	339	forward 1	TM	N in
47	LG:1082586.1:2000FEB18	248	316	forward 2	TM	N out
49	LG:1082775.1:2000FEB18	553	606	forward 1	TM	N in

50	LG:1083120.1:2000FEB18	214	291	forward 1	TM	N out
50	LG:1083120.1:2000FEB18	233	319	forward 2	TM	N out
50	LG:1083120.1:2000FEB18	252	320	forward 3	TM	N in
51	LG:1087707.1:2000FEB18	367	453	forward 1	TM	N out
51	LG:1087707.1:2000FEB18	469	531	forward 1	TM	N out
51	LG:1087707.1:2000FEB18	667	729	forward 1	TM	N out
51	LG:1087707.1:2000FEB18	742	804	forward 1	TM	N out
51	LG:1087707.1:2000FEB18	407	481	forward 2	TM	N in
51	LG:1087707.1:2000FEB18	671	739	forward 2	TM	N in
51	LG:1087707.1:2000FEB18	743	811	forward 2	TM	N in
51	LG:1087707.1:2000FEB18	570	641	forward 3	TM	N out
51	LG:1087707.1:2000FEB18	747	833	forward 3	TM	N out
52	LG:1090915.1:2000FEB18	11	61	forward 2	TM	N out
53	LG:1094230.1:2000FEB18	469	555	forward 1	TM	N out
53	LG:1094230.1:2000FEB18	449	535	forward 2	TM	N out
54	LG:474848.3:2000FEB18	445	531	forward 1	TM	N out
54	LG:474848.3:2000FEB18	456	518	forward 3	TM	N out
58	LI:236654.2:2000FEB01	221	307	forward 2	TM	N out
59	LI:200009.1:2000FEB01	1045	1131	forward 1	TM	N out
59	LI:200009.1:2000FEB01	1171	1233	forward 1	TM	N out
59	LI:200009.1:2000FEB01	1076	1162	forward 2	TM	N in
59	LI:200009.1:2000FEB01	1044	1130	forward 3	TM	N in
60	LI:758502.1:2000FEB01	286	369	forward 1	TM	N out
60	LI:758502.1:2000FEB01	755	805	forward 2	TM	N in
60	LI:758502.1:2000FEB01	780	833	forward 3	TM	N in
62	LI:789445.1:2000FEB01	9	80	forward 3	TM	N out
63	LI:789657.1:2000FEB01	854	937	forward 2	TM	N in
64	LI:789808.1:2000FEB01	347	400	forward 2	TM	N in
65	LI:792919.1:2000FEB01	176	256	forward 2	TM	
65	LI:792919.1:2000FEB01	371	427	forward 2	TM	
66	LI:793949.1:2000FEB01	208	282	forward 1	TM	N out
66	LI:793949.1:2000FEB01	472	558	forward 1	TM	N out
66	LI:793949.1:2000FEB01	455	541	forward 2	TM	N out
67	LI:794389.1:2000FEB01	265	333	forward 1	TM	N out
67	LI:794389.1:2000FEB01	424	477	forward 1	TM	N out
67	LI:794389.1:2000FEB01	384	455	forward 3	TM	N in
68	LI:796010.1:2000FEB01	351	404	forward 3	TM	N in
69	LI:796324.1:2000FEB01	365	418	forward 2	TM	N in
72	LI:798636.1:2000FEB01	490	543	forward 1	TM	N in
73	LI:800045.1:2000FEB01	627	701	forward 3	TM	N in
74	LI:800680.1:2000FEB01	334	411	forward 1	TM	N out
74	LI:800680.1:2000FEB01	359	421	forward 2	TM	N out
75	LI:800894.1:2000FEB01	536	592	forward 2	TM	N in
75	LI:800894.1:2000FEB01	300	374	forward 3	TM	N out
75	LI:800894.1:2000FEB01	396	482	forward 3	TM	N out
77	LI:801236.1:2000FEB01	262	318	forward 1	TM	N out
78	LI:803335.1:2000FEB01	412	498	forward 1	TM	N out
78	LI:803335.1:2000FEB01	423	485	forward 3	TM	N out
79	LI:803998.1:2000FEB01	221	307	forward 2	TM	N out
81	LI:808532.1:2000FEB01	472	558	forward 1	TM	N in
81	LI:808532.1:2000FEB01	117	203	forward 3	TM	N in
81	LI:808532.1:2000FEB01	363	443	forward 3	TM	N in
81	LI:808532.1:2000FEB01	558	623	forward 3	TM	N in
82	LI:443073.1:2000FEB01	293	379	forward 2	TM	N in
82	LI:443073.1:2000FEB01	81	152	forward 3	TM	N in
82	LI:443073.1:2000FEB01	189	260	forward 3	TM	N in

83	LI:479671.1:2000FEB01	523	579	forward 1	TM	N out
85	LI:810224.1:2000FEB01	246	299	forward 3	TM	
87	LG:892274.1:2000MAY19	49	105	forward 1	TM	N out
87	LG:892274.1:2000MAY19	613	681	forward 1	TM	N out
87	LG:892274.1:2000MAY19	506	589	forward 2	TM	N in
91	LG:1084051.1:2000MAY19	301	363	forward 1	TM	N in
92	LG:1076853.1:2000MAY19	964	1050	forward 1	TM	N in
92	LG:1076853.1:2000MAY19	56	130	forward 2	TM	N out
92	LG:1076853.1:2000MAY19	741	818	forward 3	TM	N in
93	LG:481631.10:2000MAY19	298	357	forward 1	TM	N out
93	LG:481631.10:2000MAY19	598	654	forward 1	TM	N out
94	LG:1088431.2:2000MAY19	379	441	forward 1	TM	N out
94	LG:1088431.2:2000MAY19	354	431	forward 3	TM	N out
95	LI:401619.10:2000MAY01	157	219	forward 1	TM	N out
95	LI:401619.10:2000MAY01	232	294	forward 1	TM	N out
95	LI:401619.10:2000MAY01	502	576	forward 1	TM	N out
95	LI:401619.10:2000MAY01	146	232	forward 2	TM	N in
95	LI:401619.10:2000MAY01	326	412	forward 2	TM	N in
95	LI:401619.10:2000MAY01	440	490	forward 2	TM	N in
95	LI:401619.10:2000MAY01	512	580	forward 2	TM	N in
95	LI:401619.10:2000MAY01	186	257	forward 3	TM	N in
95	LI:401619.10:2000MAY01	528	599	forward 3	TM	N in
96	LI:1144007.1:2000MAY01	2833	2910	forward 1	TM	N in
96	LI:1144007.1:2000MAY01	3301	3378	forward 1	TM	N in
96	LI:1144007.1:2000MAY01	3511	3597	forward 1	TM	N in
96	LI:1144007.1:2000MAY01	3634	3696	forward 1	TM	N in
96	LI:1144007.1:2000MAY01	3736	3801	forward 1	TM	N in
96	LI:1144007.1:2000MAY01	2645	2725	forward 2	TM	N out
96	LI:1144007.1:2000MAY01	2879	2965	forward 2	TM	N out
96	LI:1144007.1:2000MAY01	3356	3433	forward 2	TM	N out
96	LI:1144007.1:2000MAY01	3476	3523	forward 2	TM	N out
96	LI:1144007.1:2000MAY01	2772	2858	forward 3	TM	N in
96	LI:1144007.1:2000MAY01	3258	3332	forward 3	TM	N in
96	LI:1144007.1:2000MAY01	4017	4097	forward 3	TM	N in
97	LI:331074.1:2000MAY01	1264	1326	forward 1	TM	N in
97	LI:331074.1:2000MAY01	1357	1419	forward 1	TM	N in
97	LI:331074.1:2000MAY01	1450	1512	forward 1	TM	N in
97	LI:331074.1:2000MAY01	1540	1626	forward 1	TM	N in
97	LI:331074.1:2000MAY01	1433	1513	forward 2	TM	N in
97	LI:331074.1:2000MAY01	1574	1660	forward 2	TM	N in
97	LI:331074.1:2000MAY01	1461	1529	forward 3	TM	N in
97	LI:331074.1:2000MAY01	1560	1646	forward 3	TM	N in
98	LI:1170349.1:2000MAY01	34	102	forward 1	TM	N in
99	LG:335097.1:2000FEB18	601	672	forward 1	TM	N out
99	LG:335097.1:2000FEB18	847	909	forward 1	TM	N out
99	LG:335097.1:2000FEB18	928	981	forward 1	TM	N out
99	LG:335097.1:2000FEB18	164	244	forward 2	TM	N out
99	LG:335097.1:2000FEB18	623	682	forward 2	TM	N out
99	LG:335097.1:2000FEB18	12	74	forward 3	TM	N in
99	LG:335097.1:2000FEB18	219	299	forward 3	TM	N in
99	LG:335097.1:2000FEB18	594	680	forward 3	TM	N in
100	LG:1076451.1:2000FEB18	94	156	forward 1	TM	N in
100	LG:1076451.1:2000FEB18	101	187	forward 2	TM	N out
100	LG:1076451.1:2000FEB18	18	98	forward 3	TM	N out
100	LG:1076451.1:2000FEB18	96	164	forward 3	TM	N out
100	LG:1076451.1:2000FEB18	216	290	forward 3	TM	N out

101	LI:805478.1:2000FEB01	83	136	forward 2	TM	N out
101	LI:805478.1:2000FEB01	212	298	forward 2	TM	N out
102	LG:101269.1:2000MAY19	655	741	forward 1	TM	N in
102	LG:101269.1:2000MAY19	650	736	forward 2	TM	N in
102	LG:101269.1:2000MAY19	96	182	forward 3	TM	N in
102	LG:101269.1:2000MAY19	249	335	forward 3	TM	N in
102	LG:101269.1:2000MAY19	663	740	forward 3	TM	N in
103	LI:331087.1:2000MAY01	251	298	forward 2	TM	N out
103	LI:331087.1:2000MAY01	237	311	forward 3	TM	
104	LI:410188.1:2000MAY01	520	591	forward 1	TM	N in
104	LI:410188.1:2000MAY01	640	711	forward 1	TM	N in
104	LI:410188.1:2000MAY01	724	810	forward 1	TM	N in
104	LI:410188.1:2000MAY01	832	879	forward 1	TM	N in
104	LI:410188.1:2000MAY01	883	969	forward 1	TM	N in
104	LI:410188.1:2000MAY01	1171	1257	forward 1	TM	N in
104	LI:410188.1:2000MAY01	1303	1389	forward 1	TM	N in
104	LI:410188.1:2000MAY01	2290	2361	forward 1	TM	N in
104	LI:410188.1:2000MAY01	2389	2460	forward 1	TM	N in
104	LI:410188.1:2000MAY01	2470	2556	forward 1	TM	N in
104	LI:410188.1:2000MAY01	2635	2721	forward 1	TM	N in
104	LI:410188.1:2000MAY01	2794	2862	forward 1	TM	N in
104	LI:410188.1:2000MAY01	2878	2964	forward 1	TM	N in
104	LI:410188.1:2000MAY01	3757	3837	forward 1	TM	N in
104	LI:410188.1:2000MAY01	3871	3957	forward 1	TM	N in
104	LI:410188.1:2000MAY01	3961	4047	forward 1	TM	N in
104	LI:410188.1:2000MAY01	4111	4194	forward 1	TM	N in
104	LI:410188.1:2000MAY01	4342	4428	forward 1	TM	N in
104	LI:410188.1:2000MAY01	4492	4578	forward 1	TM	N in
104	LI:410188.1:2000MAY01	4714	4794	forward 1	TM	N in
104	LI:410188.1:2000MAY01	6439	6519	forward 1	TM	N in
104	LI:410188.1:2000MAY01	7492	7575	forward 1	TM	N in
104	LI:410188.1:2000MAY01	7783	7845	forward 1	TM	N in
104	LI:410188.1:2000MAY01	4673	4735	forward 2	TM	N in
104	LI:410188.1:2000MAY01	4766	4828	forward 2	TM	N in
104	LI:410188.1:2000MAY01	4928	5014	forward 2	TM	N in
104	LI:410188.1:2000MAY01	5231	5317	forward 2	TM	N in
104	LI:410188.1:2000MAY01	6341	6409	forward 2	TM	N in
104	LI:410188.1:2000MAY01	7655	7741	forward 2	TM	N in
104	LI:410188.1:2000MAY01	8060	8146	forward 2	TM	N in
104	LI:410188.1:2000MAY01	4776	4859	forward 3	TM	N in
104	LI:410188.1:2000MAY01	6309	6371	forward 3	TM	N in
104	LI:410188.1:2000MAY01	7704	7775	forward 3	TM	N in
105	LI:1188288.1:2000MAY01	457	519	forward 1	TM	
105	LI:1188288.1:2000MAY01	841	915	forward 1	TM	
105	LI:1188288.1:2000MAY01	958	1038	forward 1	TM	
105	LI:1188288.1:2000MAY01	1072	1140	forward 1	TM	
105	LI:1188288.1:2000MAY01	1477	1539	forward 1	TM	
105	LI:1188288.1:2000MAY01	1564	1626	forward 1	TM	
105	LI:1188288.1:2000MAY01	1810	1896	forward 1	TM	
105	LI:1188288.1:2000MAY01	2134	2220	forward 1	TM	
105	LI:1188288.1:2000MAY01	2734	2820	forward 1	TM	
105	LI:1188288.1:2000MAY01	1067	1147	forward 2	TM	N out
105	LI:1188288.1:2000MAY01	1157	1243	forward 2	TM	N out
105	LI:1188288.1:2000MAY01	1313	1399	forward 2	TM	N out
105	LI:1188288.1:2000MAY01	1556	1618	forward 2	TM	N out
105	LI:1188288.1:2000MAY01	2294	2368	forward 2	TM	N out

105	LI:1188288.1:2000MAY01	435	521	forward 3	TM	N in
105	LI:1188288.1:2000MAY01	597	683	forward 3	TM	N in
105	LI:1188288.1:2000MAY01	2301	2354	forward 3	TM	N in
105	LI:1188288.1:2000MAY01	2700	2753	forward 3	TM	N in
106	LI:427997.4:2000MAY01	148	222	forward 1	TM	N in
106	LI:427997.4:2000MAY01	745	828	forward 1	TM	N in
106	LI:427997.4:2000MAY01	1192	1278	forward 1	TM	N in
106	LI:427997.4:2000MAY01	1351	1434	forward 1	TM	N in
106	LI:427997.4:2000MAY01	1450	1518	forward 1	TM	N in
106	LI:427997.4:2000MAY01	1759	1845	forward 1	TM	N in
106	LI:427997.4:2000MAY01	134	220	forward 2	TM	N in
106	LI:427997.4:2000MAY01	749	832	forward 2	TM	N in
106	LI:427997.4:2000MAY01	1031	1087	forward 2	TM	N in
106	LI:427997.4:2000MAY01	1607	1693	forward 2	TM	N in
106	LI:427997.4:2000MAY01	1730	1816	forward 2	TM	N in
106	LI:427997.4:2000MAY01	2111	2191	forward 2	TM	N in
106	LI:427997.4:2000MAY01	150	236	forward 3	TM	N in
106	LI:427997.4:2000MAY01	681	767	forward 3	TM	N in
106	LI:427997.4:2000MAY01	765	851	forward 3	TM	N in
106	LI:427997.4:2000MAY01	1068	1124	forward 3	TM	N in
106	LI:427997.4:2000MAY01	1665	1751	forward 3	TM	N in
106	LI:427997.4:2000MAY01	1782	1856	forward 3	TM	N in
107	LG:451682.1:2000FEB18	93	155	forward 3	TM	
109	LG:481436.5:2000FEB18	583	669	forward 1	TM	N in
109	LG:481436.5:2000FEB18	769	834	forward 1	TM	N in
109	LG:481436.5:2000FEB18	1111	1176	forward 1	TM	N in
109	LG:481436.5:2000FEB18	575	655	forward 2	TM	N out
109	LG:481436.5:2000FEB18	764	826	forward 2	TM	N out
109	LG:481436.5:2000FEB18	1091	1153	forward 2	TM	N out
109	LG:481436.5:2000FEB18	1187	1249	forward 2	TM	N out
109	LG:481436.5:2000FEB18	84	170	forward 3	TM	N in
109	LG:481436.5:2000FEB18	753	833	forward 3	TM	N in
109	LG:481436.5:2000FEB18	1164	1241	forward 3	TM	N in
110	LI:793701.1:2000FEB01	352	405	forward 1	TM	N in
110	LI:793701.1:2000FEB01	389	475	forward 2	TM	N in
111	LI:373637.1:2000FEB01	412	498	forward 1	TM	
111	LI:373637.1:2000FEB01	434	520	forward 2	TM	N out
111	LI:373637.1:2000FEB01	866	919	forward 2	TM	N out
111	LI:373637.1:2000FEB01	423	473	forward 3	TM	N in
111	LI:373637.1:2000FEB01	867	920	forward 3	TM	N in
112	LG:239368.2:2000MAY19	241	327	forward 1	TM	N out
113	LI:053826.1:2000MAY01	31	117	forward 1	TM	N out
113	LI:053826.1:2000MAY01	1102	1188	forward 1	TM	N out
113	LI:053826.1:2000MAY01	1282	1350	forward 1	TM	N out
113	LI:053826.1:2000MAY01	41	112	forward 2	TM	N out
113	LI:053826.1:2000MAY01	164	238	forward 2	TM	N out
113	LI:053826.1:2000MAY01	461	538	forward 2	TM	N out
113	LI:053826.1:2000MAY01	1130	1192	forward 2	TM	N out
113	LI:053826.1:2000MAY01	1214	1276	forward 2	TM	N out
113	LI:053826.1:2000MAY01	1307	1378	forward 2	TM	N out
113	LI:053826.1:2000MAY01	126	200	forward 3	TM	N in
113	LI:053826.1:2000MAY01	348	416	forward 3	TM	N in
113	LI:053826.1:2000MAY01	624	683	forward 3	TM	N in
113	LI:053826.1:2000MAY01	1215	1277	forward 3	TM	N in
113	LI:053826.1:2000MAY01	1290	1352	forward 3	TM	N in
115	LI:1071427.96:2000MAY01	1072	1140	forward 1	TM	

115	LI:1071427.96:2000MAY01	1297	1383	forward 1	TM	
115	LI:1071427.96:2000MAY01	1459	1536	forward 1	TM	
115	LI:1071427.96:2000MAY01	1765	1851	forward 1	TM	
115	LI:1071427.96:2000MAY01	1909	1971	forward 1	TM	
115	LI:1071427.96:2000MAY01	2002	2064	forward 1	TM	
115	LI:1071427.96:2000MAY01	1562	1648	forward 2	TM	N out
115	LI:1071427.96:2000MAY01	1706	1792	forward 2	TM	N out
115	LI:1071427.96:2000MAY01	1823	1885	forward 2	TM	N out
115	LI:1071427.96:2000MAY01	1913	1975	forward 2	TM	N out
115	LI:1071427.96:2000MAY01	2045	2098	forward 2	TM	N out
115	LI:1071427.96:2000MAY01	384	470	forward 3	TM	N out
115	LI:1071427.96:2000MAY01	840	926	forward 3	TM	N out
115	LI:1071427.96:2000MAY01	987	1049	forward 3	TM	N out
115	LI:1071427.96:2000MAY01	1092	1154	forward 3	TM	N out
115	LI:1071427.96:2000MAY01	1383	1454	forward 3	TM	N out
115	LI:1071427.96:2000MAY01	1599	1655	forward 3	TM	N out
115	LI:1071427.96:2000MAY01	1767	1844	forward 3	TM	N out
115	LI:1071427.96:2000MAY01	1884	1952	forward 3	TM	N out
115	LI:1071427.96:2000MAY01	2013	2099	forward 3	TM	N out
115	LI:1071427.96:2000MAY01	2127	2189	forward 3	TM	N out
116	LI:336338.8:2000MAY01	100	186	forward 1	TM	N out
116	LI:336338.8:2000MAY01	427	513	forward 1	TM	N out
116	LI:336338.8:2000MAY01	110	196	forward 2	TM	
116	LI:336338.8:2000MAY01	281	367	forward 2	TM	
116	LI:336338.8:2000MAY01	422	508	forward 2	TM	
116	LI:336338.8:2000MAY01	354	416	forward 3	TM	N out
116	LI:336338.8:2000MAY01	432	494	forward 3	TM	N out
117	LG:345527.1:2000FEB18	46	120	forward 1	TM	N out
117	LG:345527.1:2000FEB18	917	979	forward 2	TM	N out
117	LG:345527.1:2000FEB18	1010	1072	forward 2	TM	N out
117	LG:345527.1:2000FEB18	1112	1198	forward 2	TM	N out
117	LG:345527.1:2000FEB18	96	182	forward 3	TM	N out
117	LG:345527.1:2000FEB18	474	536	forward 3	TM	N out
117	LG:345527.1:2000FEB18	552	614	forward 3	TM	N out
118	LG:1089383.1:2000FEB18	43	126	forward 1	TM	N out
118	LG:1089383.1:2000FEB18	14	100	forward 2	TM	
118	LG:1089383.1:2000FEB18	140	205	forward 2	TM	
118	LG:1089383.1:2000FEB18	12	59	forward 3	TM	N out
120	LG:1093216.1:2000FEB18	31	117	forward 1	TM	N out
120	LG:1093216.1:2000FEB18	151	234	forward 1	TM	N out
120	LG:1093216.1:2000FEB18	283	348	forward 1	TM	N out
120	LG:1093216.1:2000FEB18	23	109	forward 2	TM	N in
120	LG:1093216.1:2000FEB18	143	193	forward 2	TM	N in
120	LG:1093216.1:2000FEB18	48	122	forward 3	TM	N out
120	LG:1093216.1:2000FEB18	180	263	forward 3	TM	N out
122	LI:335671.2:2000FEB01	22	108	forward 1	TM	N out
122	LI:335671.2:2000FEB01	1048	1134	forward 1	TM	N out
122	LI:335671.2:2000FEB01	854	916	forward 2	TM	N in
122	LI:335671.2:2000FEB01	926	988	forward 2	TM	N in
122	LI:335671.2:2000FEB01	998	1072	forward 2	TM	N in
122	LI:335671.2:2000FEB01	399	461	forward 3	TM	N out
122	LI:335671.2:2000FEB01	480	542	forward 3	TM	N out
122	LI:335671.2:2000FEB01	576	662	forward 3	TM	N out
122	LI:335671.2:2000FEB01	1023	1085	forward 3	TM	N out
122	LI:335671.2:2000FEB01	1098	1160	forward 3	TM	N out
122	LI:335671.2:2000FEB01	1173	1235	forward 3	TM	N out

123	LI:793758.1:2000FEB01	31	117	forward 1	TM	N out
123	LI:793758.1:2000FEB01	151	234	forward 1	TM	N out
123	LI:793758.1:2000FEB01	283	348	forward 1	TM	N out
123	LI:793758.1:2000FEB01	23	109	forward 2	TM	N in
123	LI:793758.1:2000FEB01	143	193	forward 2	TM	N in
123	LI:793758.1:2000FEB01	48	122	forward 3	TM	N out
123	LI:793758.1:2000FEB01	180	263	forward 3	TM	N out
124	LI:803718.1:2000FEB01	43	126	forward 1	TM	N out
124	LI:803718.1:2000FEB01	14	100	forward 2	TM	
124	LI:803718.1:2000FEB01	140	205	forward 2	TM	
124	LI:803718.1:2000FEB01	12	59	forward 3	TM	N out
125	LI:412179.1:2000FEB01	328	414	forward 1	TM	
125	LI:412179.1:2000FEB01	436	504	forward 1	TM	
125	LI:412179.1:2000FEB01	56	115	forward 2	TM	N out
125	LI:412179.1:2000FEB01	413	475	forward 2	TM	N out
125	LI:412179.1:2000FEB01	512	574	forward 2	TM	N out
125	LI:412179.1:2000FEB01	96	176	forward 3	TM	N out
125	LI:412179.1:2000FEB01	384	446	forward 3	TM	N out
125	LI:412179.1:2000FEB01	462	524	forward 3	TM	N out
126	LI:815679.1:2000FEB01	10	84	forward 1	TM	N out
126	LI:815679.1:2000FEB01	313	399	forward 1	TM	N out
126	LI:815679.1:2000FEB01	946	1032	forward 1	TM	N out
126	LI:815679.1:2000FEB01	1171	1248	forward 1	TM	N out
126	LI:815679.1:2000FEB01	323	409	forward 2	TM	N in
126	LI:815679.1:2000FEB01	500	568	forward 2	TM	N in
126	LI:815679.1:2000FEB01	971	1021	forward 2	TM	N in
126	LI:815679.1:2000FEB01	1493	1561	forward 2	TM	N in
126	LI:815679.1:2000FEB01	15	92	forward 3	TM	N in
126	LI:815679.1:2000FEB01	285	356	forward 3	TM	N in
126	LI:815679.1:2000FEB01	690	764	forward 3	TM	N in
126	LI:815679.1:2000FEB01	993	1076	forward 3	TM	N in
126	LI:815679.1:2000FEB01	1626	1712	forward 3	TM	N in
127	LI:481361.3:2000FEB01	199	252	forward 1	TM	N out
128	LG:247388.1:2000MAY19	190	240	forward 1	TM	N out
128	LG:247388.1:2000MAY19	233	319	forward 2	TM	N out
128	LG:247388.1:2000MAY19	446	532	forward 2	TM	N out
130	LI:787618.1:2000MAY01	10	84	forward 1	TM	N in
130	LI:787618.1:2000MAY01	313	399	forward 1	TM	N in
130	LI:787618.1:2000MAY01	679	750	forward 1	TM	N in
130	LI:787618.1:2000MAY01	1018	1098	forward 1	TM	N in
130	LI:787618.1:2000MAY01	1189	1266	forward 1	TM	N in
130	LI:787618.1:2000MAY01	323	409	forward 2	TM	N out
130	LI:787618.1:2000MAY01	500	568	forward 2	TM	N out
130	LI:787618.1:2000MAY01	944	1030	forward 2	TM	N out
130	LI:787618.1:2000MAY01	1508	1582	forward 2	TM	N out
130	LI:787618.1:2000MAY01	1616	1702	forward 2	TM	N out
130	LI:787618.1:2000MAY01	15	92	forward 3	TM	N out
130	LI:787618.1:2000MAY01	285	356	forward 3	TM	N out
131	LI:331610.2:2000MAY01	91	156	forward 1	TM	
131	LI:331610.2:2000MAY01	277	363	forward 1	TM	
131	LI:331610.2:2000MAY01	682	744	forward 1	TM	
131	LI:331610.2:2000MAY01	4126	4212	forward 1	TM	
131	LI:331610.2:2000MAY01	4951	5001	forward 1	TM	
131	LI:331610.2:2000MAY01	5023	5109	forward 1	TM	
131	LI:331610.2:2000MAY01	5128	5190	forward 1	TM	
131	LI:331610.2:2000MAY01	5407	5469	forward 1	TM	

131	LI:331610.2:2000MAY01	5485	5547	forward 1	TM	
131	LI:331610.2:2000MAY01	5563	5625	forward 1	TM	
131	LI:331610.2:2000MAY01	5728	5805	forward 1	TM	
131	LI:331610.2:2000MAY01	5896	5949	forward 1	TM	
131	LI:331610.2:2000MAY01	6268	6327	forward 1	TM	
131	LI:331610.2:2000MAY01	6454	6522	forward 1	TM	
131	LI:331610.2:2000MAY01	6559	6645	forward 1	TM	
131	LI:331610.2:2000MAY01	7477	7539	forward 1	TM	
131	LI:331610.2:2000MAY01	7552	7614	forward 1	TM	
131	LI:331610.2:2000MAY01	671	724	forward 2	TM	N out
131	LI:331610.2:2000MAY01	4127	4213	forward 2	TM	N out
131	LI:331610.2:2000MAY01	4928	5011	forward 2	TM	N out
131	LI:331610.2:2000MAY01	5051	5113	forward 2	TM	N out
131	LI:331610.2:2000MAY01	5135	5197	forward 2	TM	N out
131	LI:331610.2:2000MAY01	5207	5269	forward 2	TM	N out
131	LI:331610.2:2000MAY01	5537	5611	forward 2	TM	N out
131	LI:331610.2:2000MAY01	5726	5797	forward 2	TM	N out
131	LI:331610.2:2000MAY01	5903	5989	forward 2	TM	N out
131	LI:331610.2:2000MAY01	6392	6478	forward 2	TM	N out
131	LI:331610.2:2000MAY01	6746	6814	forward 2	TM	N out
131	LI:331610.2:2000MAY01	7295	7381	forward 2	TM	N out
131	LI:331610.2:2000MAY01	7586	7633	forward 2	TM	N out
131	LI:331610.2:2000MAY01	2763	2849	forward 3	TM	
131	LI:331610.2:2000MAY01	4527	4595	forward 3	TM	
131	LI:331610.2:2000MAY01	5079	5165	forward 3	TM	
131	LI:331610.2:2000MAY01	5445	5516	forward 3	TM	
131	LI:331610.2:2000MAY01	5676	5759	forward 3	TM	
131	LI:331610.2:2000MAY01	6255	6341	forward 3	TM	
131	LI:331610.2:2000MAY01	6378	6464	forward 3	TM	
131	LI:331610.2:2000MAY01	6624	6692	forward 3	TM	
131	LI:331610.2:2000MAY01	6705	6779	forward 3	TM	
131	LI:331610.2:2000MAY01	6810	6884	forward 3	TM	
131	LI:331610.2:2000MAY01	7062	7133	forward 3	TM	
131	LI:331610.2:2000MAY01	7677	7748	forward 3	TM	
131	LI:331610.2:2000MAY01	7833	7919	forward 3	TM	
132	LG:982697.1:2000FEB18	355	441	forward 1	TM	N in
132	LG:982697.1:2000FEB18	946	993	forward 1	TM	N in
132	LG:982697.1:2000FEB18	897	983	forward 3	TM	N in
132	LG:982697.1:2000FEB18	1215	1301	forward 3	TM	N in
133	LG:1080896.1:2000FEB18	367	426	forward 1	TM	N in
133	LG:1080896.1:2000FEB18	476	562	forward 2	TM	N in
133	LG:1080896.1:2000FEB18	815	901	forward 2	TM	N in
133	LG:1080896.1:2000FEB18	342	395	forward 3	TM	N in
134	LI:811341.1:2000FEB01	562	615	forward 1	TM	N out
134	LI:811341.1:2000FEB01	691	777	forward 1	TM	N out
135	LI:903225.1:2000FEB01	20	100	forward 2	TM	N out
135	LI:903225.1:2000FEB01	12	83	forward 3	TM	N out
135	LI:903225.1:2000FEB01	768	827	forward 3	TM	N out
137	LG:979580.1:2000MAY19	298	354	forward 1	TM	N in
137	LG:979580.1:2000MAY19	826	909	forward 1	TM	N in
137	LG:979580.1:2000MAY19	934	1020	forward 1	TM	N in
137	LG:979580.1:2000MAY19	233	289	forward 2	TM	N out
137	LG:979580.1:2000MAY19	338	418	forward 2	TM	N out
137	LG:979580.1:2000MAY19	201	272	forward 3	TM	N in
138	LI:1169865.1:2000MAY01	197	283	forward 2	TM	N in
138	LI:1169865.1:2000MAY01	863	949	forward 2	TM	N in

139	LG:337818.2:2000FEB18	40	117	forward 1	TM	N out
139	LG:337818.2:2000FEB18	532	618	forward 1	TM	N out
139	LG:337818.2:2000FEB18	907	993	forward 1	TM	N out
139	LG:337818.2:2000FEB18	1372	1425	forward 1	TM	N out
140	LI:337818.1:2000FEB01	40	114	forward 1	TM	N in
140	LI:337818.1:2000FEB01	401	466	forward 2	TM	N in
140	LI:337818.1:2000FEB01	852	905	forward 3	TM	N in
141	LG:241577.4:2000MAY19	496	582	forward 1	TM	N in
142	LG:344786.4:2000MAY19	19	105	forward 1	TM	N out
142	LG:344786.4:2000MAY19	14	88	forward 2	TM	N in
142	LG:344786.4:2000MAY19	173	247	forward 2	TM	N in
142	LG:344786.4:2000MAY19	21	107	forward 3	TM	
143	LI:414307.1:2000FEB01	116	202	forward 2	TM	N in
144	LI:202943.2:2000FEB01	166	237	forward 1	TM	N in
144	LI:202943.2:2000FEB01	263	313	forward 2	TM	N out
144	LI:202943.2:2000FEB01	276	326	forward 3	TM	N in
146	LI:815961.1:2000FEB01	232	291	forward 1	TM	N out
146	LI:815961.1:2000FEB01	81	167	forward 3	TM	N out
146	LI:815961.1:2000FEB01	243	329	forward 3	TM	N out
146	LI:815961.1:2000FEB01	354	422	forward 3	TM	N out
146	LI:815961.1:2000FEB01	573	659	forward 3	TM	N out
146	LI:815961.1:2000FEB01	741	803	forward 3	TM	N out
147	LG:120744.1:2000MAY19	181	249	forward 1	TM	N out
147	LG:120744.1:2000MAY19	188	256	forward 2	TM	
147	LG:120744.1:2000MAY19	275	328	forward 2	TM	
148	LI:757520.1:2000MAY01	2140	2220	forward 1	TM	N in
148	LI:757520.1:2000MAY01	2293	2379	forward 1	TM	N in
148	LI:757520.1:2000MAY01	1988	2059	forward 2	TM	N in
148	LI:757520.1:2000MAY01	2285	2359	forward 2	TM	N in
148	LI:757520.1:2000MAY01	1677	1763	forward 3	TM	
148	LI:757520.1:2000MAY01	1995	2066	forward 3	TM	
149	LG:160570.1:2000FEB18	345	413	forward 3	TM	N out
149	LG:160570.1:2000FEB18	462	518	forward 3	TM	N out
151	LI:221285.1:2000FEB01	1375	1452	forward 1	TM	N out
152	LI:401605.2:2000FEB01	235	321	forward 1	TM	N in
152	LI:401605.2:2000FEB01	192	263	forward 3	TM	N in
152	LI:401605.2:2000FEB01	489	563	forward 3	TM	N in
153	LI:329017.1:2000FEB01	179	235	forward 2	TM	N in
153	LI:329017.1:2000FEB01	359	433	forward 2	TM	N in
153	LI:329017.1:2000FEB01	449	526	forward 2	TM	N in
153	LI:329017.1:2000FEB01	617	703	forward 2	TM	N in
153	LI:329017.1:2000FEB01	920	973	forward 2	TM	N in
155	LG:403409.1:2000MAY19	136	222	forward 1	TM	N out
155	LG:403409.1:2000MAY19	973	1029	forward 1	TM	N out
155	LG:403409.1:2000MAY19	1285	1371	forward 1	TM	N out
155	LG:403409.1:2000MAY19	182	268	forward 2	TM	N in
156	LG:233933.5:2000MAY19	148	234	forward 1	TM	N out
156	LG:233933.5:2000MAY19	39	125	forward 3	TM	N out
157	LI:290344.1:2000MAY01	232	312	forward 1	TM	N out
157	LI:290344.1:2000MAY01	1258	1311	forward 1	TM	N out
157	LI:290344.1:2000MAY01	3640	3714	forward 1	TM	N out
157	LI:290344.1:2000MAY01	4366	4449	forward 1	TM	N out
157	LI:290344.1:2000MAY01	4468	4548	forward 1	TM	N out
157	LI:290344.1:2000MAY01	146	226	forward 2	TM	N out
157	LI:290344.1:2000MAY01	3122	3196	forward 2	TM	N out
157	LI:290344.1:2000MAY01	3833	3919	forward 2	TM	N out

157	LI:290344.1:2000MAY01	4457	4537	forward 2	TM	N out
157	LI:290344.1:2000MAY01	4760	4846	forward 2	TM	N out
157	LI:290344.1:2000MAY01	432	503	forward 3	TM	N out
157	LI:290344.1:2000MAY01	1647	1733	forward 3	TM	N out
157	LI:290344.1:2000MAY01	3177	3248	forward 3	TM	N out
157	LI:290344.1:2000MAY01	3594	3680	forward 3	TM	N out
157	LI:290344.1:2000MAY01	3753	3815	forward 3	TM	N out
157	LI:290344.1:2000MAY01	3864	3926	forward 3	TM	N out
157	LI:290344.1:2000MAY01	4443	4526	forward 3	TM	N out
158	LI:410742.1:2000MAY01	136	210	forward 1	TM	N out
158	LI:410742.1:2000MAY01	2200	2286	forward 1	TM	N out
158	LI:410742.1:2000MAY01	2437	2514	forward 1	TM	N out
158	LI:410742.1:2000MAY01	3149	3229	forward 2	TM	N in
158	LI:410742.1:2000MAY01	3437	3505	forward 2	TM	N in
158	LI:410742.1:2000MAY01	510	578	forward 3	TM	N in
158	LI:410742.1:2000MAY01	1905	1991	forward 3	TM	N in
158	LI:410742.1:2000MAY01	2811	2897	forward 3	TM	N in
158	LI:410742.1:2000MAY01	3168	3254	forward 3	TM	N in
159	LG:406568.1:2000MAY19	490	549	forward 1	TM	N in
159	LG:406568.1:2000MAY19	1732	1818	forward 1	TM	N in
159	LG:406568.1:2000MAY19	1825	1899	forward 1	TM	N in
159	LG:406568.1:2000MAY19	1918	2004	forward 1	TM	N in
159	LG:406568.1:2000MAY19	12	59	forward 3	TM	N in
159	LG:406568.1:2000MAY19	1935	2018	forward 3	TM	N in
159	LG:406568.1:2000MAY19	2094	2174	forward 3	TM	N in
160	LI:283762.1:2000MAY01	1675	1746	forward 1	TM	
160	LI:283762.1:2000MAY01	2095	2181	forward 1	TM	
160	LI:283762.1:2000MAY01	2632	2718	forward 1	TM	
160	LI:283762.1:2000MAY01	2830	2916	forward 1	TM	
160	LI:283762.1:2000MAY01	2941	3027	forward 1	TM	
160	LI:283762.1:2000MAY01	3235	3321	forward 1	TM	
160	LI:283762.1:2000MAY01	3328	3414	forward 1	TM	
160	LI:283762.1:2000MAY01	3592	3666	forward 1	TM	
160	LI:283762.1:2000MAY01	3682	3768	forward 1	TM	
160	LI:283762.1:2000MAY01	4153	4224	forward 1	TM	
160	LI:283762.1:2000MAY01	4360	4434	forward 1	TM	
160	LI:283762.1:2000MAY01	4594	4656	forward 1	TM	
160	LI:283762.1:2000MAY01	4681	4743	forward 1	TM	
160	LI:283762.1:2000MAY01	4885	4962	forward 1	TM	
160	LI:283762.1:2000MAY01	5011	5061	forward 1	TM	
160	LI:283762.1:2000MAY01	92	178	forward 2	TM	N in
160	LI:283762.1:2000MAY01	278	364	forward 2	TM	N in
160	LI:283762.1:2000MAY01	995	1075	forward 2	TM	N in
160	LI:283762.1:2000MAY01	1523	1597	forward 2	TM	N in
160	LI:283762.1:2000MAY01	1817	1903	forward 2	TM	N in
160	LI:283762.1:2000MAY01	2522	2599	forward 2	TM	N in
160	LI:283762.1:2000MAY01	2666	2752	forward 2	TM	N in
160	LI:283762.1:2000MAY01	2837	2887	forward 2	TM	N in
160	LI:283762.1:2000MAY01	3038	3097	forward 2	TM	N in
160	LI:283762.1:2000MAY01	3563	3625	forward 2	TM	N in
160	LI:283762.1:2000MAY01	3638	3700	forward 2	TM	N in
160	LI:283762.1:2000MAY01	4067	4144	forward 2	TM	N in
160	LI:283762.1:2000MAY01	4439	4522	forward 2	TM	N in
160	LI:283762.1:2000MAY01	4685	4765	forward 2	TM	N in
160	LI:283762.1:2000MAY01	4784	4843	forward 2	TM	N in
160	LI:283762.1:2000MAY01	4973	5050	forward 2	TM	N in

160	LI:283762.1:2000MAY01	5072	5125	forward 2	TM	N in
160	LI:283762.1:2000MAY01	693	755	forward 3	TM	N out
160	LI:283762.1:2000MAY01	765	827	forward 3	TM	N out
160	LI:283762.1:2000MAY01	840	902	forward 3	TM	N out
160	LI:283762.1:2000MAY01	1623	1694	forward 3	TM	N out
160	LI:283762.1:2000MAY01	1800	1880	forward 3	TM	N out
160	LI:283762.1:2000MAY01	2622	2708	forward 3	TM	N out
160	LI:283762.1:2000MAY01	2778	2861	forward 3	TM	N out
160	LI:283762.1:2000MAY01	3144	3230	forward 3	TM	N out
160	LI:283762.1:2000MAY01	3276	3362	forward 3	TM	N out
160	LI:283762.1:2000MAY01	3441	3527	forward 3	TM	N out
160	LI:283762.1:2000MAY01	3666	3752	forward 3	TM	N out
160	LI:283762.1:2000MAY01	4077	4163	forward 3	TM	N out
160	LI:283762.1:2000MAY01	4245	4331	forward 3	TM	N out
160	LI:283762.1:2000MAY01	4395	4481	forward 3	TM	N out
160	LI:283762.1:2000MAY01	4584	4646	forward 3	TM	N out
160	LI:283762.1:2000MAY01	4662	4724	forward 3	TM	N out
160	LI:283762.1:2000MAY01	4845	4892	forward 3	TM	N out
161	LI:347687.113:2000MAY01	319	405	forward 1	TM	N out
161	LI:347687.113:2000MAY01	463	549	forward 1	TM	N out
161	LI:347687.113:2000MAY01	733	819	forward 1	TM	N out
161	LI:347687.113:2000MAY01	1240	1293	forward 1	TM	N out
161	LI:347687.113:2000MAY01	1720	1797	forward 1	TM	N out
161	LI:347687.113:2000MAY01	1861	1908	forward 1	TM	N out
161	LI:347687.113:2000MAY01	1972	2034	forward 1	TM	N out
161	LI:347687.113:2000MAY01	2050	2112	forward 1	TM	N out
161	LI:347687.113:2000MAY01	2308	2394	forward 1	TM	N out
161	LI:347687.113:2000MAY01	977	1057	forward 2	TM	N in
161	LI:347687.113:2000MAY01	1250	1309	forward 2	TM	N in
161	LI:347687.113:2000MAY01	1730	1792	forward 2	TM	N in
161	LI:347687.113:2000MAY01	1808	1870	forward 2	TM	N in
161	LI:347687.113:2000MAY01	1886	1948	forward 2	TM	N in
161	LI:347687.113:2000MAY01	324	398	forward 3	TM	N in
161	LI:347687.113:2000MAY01	948	1034	forward 3	TM	N in
161	LI:347687.113:2000MAY01	1686	1763	forward 3	TM	N in
161	LI:347687.113:2000MAY01	1791	1874	forward 3	TM	N in
161	LI:347687.113:2000MAY01	2025	2108	forward 3	TM	N in
163	LG:451710.1:2000FEB18	502	588	forward 1	TM	N in
163	LG:451710.1:2000FEB18	453	515	forward 3	TM	N in
164	LG:455771.1:2000FEB18	199	285	forward 1	TM	N out
165	LG:452089.1:2000FEB18	695	772	forward 2	TM	N out
165	LG:452089.1:2000FEB18	708	764	forward 3	TM	N out
166	LG:246415.1:2000FEB18	196	246	forward 1	TM	N in
167	LG:414144.10:2000FEB18	589	672	forward 1	TM	N in
167	LG:414144.10:2000FEB18	615	692	forward 3	TM	N out
168	LG:1101445.1:2000FEB18	787	858	forward 1	TM	N out
168	LG:1101445.1:2000FEB18	506	592	forward 2	TM	N out
169	LG:452134.1:2000FEB18	276	326	forward 3	TM	N out
170	LI:903021.1:2000FEB01	109	162	forward 1	TM	N out
172	LG:449404.1:2000MAY19	163	219	forward 1	TM	N out
172	LG:449404.1:2000MAY19	200	280	forward 2	TM	N out
173	LG:449413.1:2000MAY19	353	439	forward 2	TM	N out
177	LG:1101153.1:2000MAY19	520	600	forward 1	TM	N in
177	LG:1101153.1:2000MAY19	585	671	forward 3	TM	N in
178	LI:257695.20:2000MAY01	433	516	forward 1	TM	N in
179	LI:455771.1:2000MAY01	199	285	forward 1	TM	N out

180	LI:274551.1:2000MAY01	81	152	forward 3	TM	N out
180	LI:274551.1:2000MAY01	216	269	forward 3	TM	N out
181	LI:035973.1:2000MAY01	622	708	forward 1	TM	N out
181	LI:035973.1:2000MAY01	596	682	forward 2	TM	N out
181	LI:035973.1:2000MAY01	588	674	forward 3	TM	N out
182	LG:978427.5:2000FEB18	221	295	forward 2	TM	N out
182	LG:978427.5:2000FEB18	365	433	forward 2	TM	N out
182	LG:978427.5:2000FEB18	198	284	forward 3	TM	N out
183	LG:247781.2:2000FEB18	22	108	forward 1	TM	N in
183	LG:247781.2:2000FEB18	1114	1200	forward 1	TM	N in
183	LG:247781.2:2000FEB18	1149	1235	forward 3	TM	N in
185	LI:333307.2:2000FEB01	24	98	forward 3	TM	N out
187	LG:414732.1:2000MAY19	40	93	forward 1	TM	N out
187	LG:414732.1:2000MAY19	156	233	forward 3	TM	N out
188	LG:413910.6:2000MAY19	385	441	forward 1	TM	N out
188	LG:413910.6:2000MAY19	886	948	forward 1	TM	N out
188	LG:413910.6:2000MAY19	104	190	forward 2	TM	N out
188	LG:413910.6:2000MAY19	387	461	forward 3	TM	N out
188	LG:413910.6:2000MAY19	921	1007	forward 3	TM	N out
189	LI:414732.2:2000MAY01	34	93	forward 1	TM	N out
189	LI:414732.2:2000MAY01	24	110	forward 3	TM	N out
189	LI:414732.2:2000MAY01	159	236	forward 3	TM	N out
190	LI:900264.2:2000MAY01	730	807	forward 1	TM	N in
190	LI:900264.2:2000MAY01	1018	1092	forward 1	TM	N in
190	LI:900264.2:2000MAY01	1294	1350	forward 1	TM	N in
190	LI:900264.2:2000MAY01	1519	1578	forward 1	TM	N in
190	LI:900264.2:2000MAY01	2311	2397	forward 1	TM	N in
190	LI:900264.2:2000MAY01	2509	2562	forward 1	TM	N in
190	LI:900264.2:2000MAY01	2752	2808	forward 1	TM	N in
190	LI:900264.2:2000MAY01	3103	3165	forward 1	TM	N in
190	LI:900264.2:2000MAY01	3178	3240	forward 1	TM	N in
190	LI:900264.2:2000MAY01	3253	3315	forward 1	TM	N in
190	LI:900264.2:2000MAY01	3424	3510	forward 1	TM	N in
190	LI:900264.2:2000MAY01	3520	3603	forward 1	TM	N in
190	LI:900264.2:2000MAY01	3883	3945	forward 1	TM	N in
190	LI:900264.2:2000MAY01	3982	4044	forward 1	TM	N in
190	LI:900264.2:2000MAY01	68	154	forward 2	TM	
190	LI:900264.2:2000MAY01	188	274	forward 2	TM	
190	LI:900264.2:2000MAY01	1079	1165	forward 2	TM	
190	LI:900264.2:2000MAY01	2285	2359	forward 2	TM	
190	LI:900264.2:2000MAY01	2732	2812	forward 2	TM	
190	LI:900264.2:2000MAY01	3095	3172	forward 2	TM	
190	LI:900264.2:2000MAY01	3260	3319	forward 2	TM	
190	LI:900264.2:2000MAY01	3434	3505	forward 2	TM	
190	LI:900264.2:2000MAY01	3515	3601	forward 2	TM	
190	LI:900264.2:2000MAY01	3662	3748	forward 2	TM	
190	LI:900264.2:2000MAY01	3842	3913	forward 2	TM	
190	LI:900264.2:2000MAY01	3992	4063	forward 2	TM	
190	LI:900264.2:2000MAY01	198	248	forward 3	TM	N in
190	LI:900264.2:2000MAY01	1080	1133	forward 3	TM	N in
190	LI:900264.2:2000MAY01	1431	1517	forward 3	TM	N in
190	LI:900264.2:2000MAY01	1518	1571	forward 3	TM	N in
190	LI:900264.2:2000MAY01	1740	1814	forward 3	TM	N in
190	LI:900264.2:2000MAY01	2409	2480	forward 3	TM	N in
190	LI:900264.2:2000MAY01	2928	2993	forward 3	TM	N in
190	LI:900264.2:2000MAY01	3096	3161	forward 3	TM	N in

190	LI:900264.2:2000MAY01	3342	3404	forward 3	TM	N in
190	LI:900264.2:2000MAY01	3447	3509	forward 3	TM	N in
190	LI:900264.2:2000MAY01	3531	3614	forward 3	TM	N in
190	LI:900264.2:2000MAY01	3987	4064	forward 3	TM	N in
191	LI:335593.1:2000MAY01	685	771	forward 1	TM	N in
191	LI:335593.1:2000MAY01	1273	1335	forward 1	TM	N in
191	LI:335593.1:2000MAY01	1366	1428	forward 1	TM	N in
191	LI:335593.1:2000MAY01	710	757	forward 2	TM	N in
191	LI:335593.1:2000MAY01	1250	1336	forward 2	TM	N in
191	LI:335593.1:2000MAY01	1358	1408	forward 2	TM	N in
191	LI:335593.1:2000MAY01	1448	1525	forward 2	TM	N in
191	LI:335593.1:2000MAY01	1604	1690	forward 2	TM	N in
191	LI:335593.1:2000MAY01	81	128	forward 3	TM	N in
191	LI:335593.1:2000MAY01	246	296	forward 3	TM	N in
191	LI:335593.1:2000MAY01	807	866	forward 3	TM	N in
191	LI:335593.1:2000MAY01	876	947	forward 3	TM	N in
191	LI:335593.1:2000MAY01	1155	1217	forward 3	TM	N in
191	LI:335593.1:2000MAY01	1233	1295	forward 3	TM	N in
191	LI:335593.1:2000MAY01	1359	1445	forward 3	TM	N in
192	LI:1189543.1:2000MAY01	1765	1842	forward 1	TM	
192	LI:1189543.1:2000MAY01	1861	1935	forward 1	TM	
192	LI:1189543.1:2000MAY01	2236	2307	forward 1	TM	
192	LI:1189543.1:2000MAY01	2356	2442	forward 1	TM	
192	LI:1189543.1:2000MAY01	2476	2544	forward 1	TM	
192	LI:1189543.1:2000MAY01	2659	2712	forward 1	TM	
192	LI:1189543.1:2000MAY01	3097	3174	forward 1	TM	
192	LI:1189543.1:2000MAY01	3217	3288	forward 1	TM	
192	LI:1189543.1:2000MAY01	3439	3492	forward 1	TM	
192	LI:1189543.1:2000MAY01	860	946	forward 2	TM	
192	LI:1189543.1:2000MAY01	1016	1099	forward 2	TM	
192	LI:1189543.1:2000MAY01	1145	1216	forward 2	TM	
192	LI:1189543.1:2000MAY01	1601	1672	forward 2	TM	
192	LI:1189543.1:2000MAY01	1691	1768	forward 2	TM	
192	LI:1189543.1:2000MAY01	2411	2485	forward 2	TM	
192	LI:1189543.1:2000MAY01	2831	2917	forward 2	TM	
192	LI:1189543.1:2000MAY01	3080	3166	forward 2	TM	
192	LI:1189543.1:2000MAY01	3227	3310	forward 2	TM	
192	LI:1189543.1:2000MAY01	1155	1229	forward 3	TM	N out
192	LI:1189543.1:2000MAY01	1683	1766	forward 3	TM	N out
192	LI:1189543.1:2000MAY01	1770	1838	forward 3	TM	N out
192	LI:1189543.1:2000MAY01	2019	2069	forward 3	TM	N out
192	LI:1189543.1:2000MAY01	2352	2438	forward 3	TM	N out
192	LI:1189543.1:2000MAY01	2508	2594	forward 3	TM	N out
192	LI:1189543.1:2000MAY01	3030	3101	forward 3	TM	N out
192	LI:1189543.1:2000MAY01	3183	3263	forward 3	TM	N out
192	LI:1189543.1:2000MAY01	3360	3446	forward 3	TM	N out
193	LG:455450.1:2000FEB18	422	490	forward 2	TM	N out
194	LG:1040978.1:2000FEB18	500	586	forward 2	TM	N out
194	LG:1040978.1:2000FEB18	276	332	forward 3	TM	N out
196	LG:132147.3:2000FEB18	259	345	forward 1	TM	N out
196	LG:132147.3:2000FEB18	418	504	forward 1	TM	N out
196	LG:132147.3:2000FEB18	718	780	forward 1	TM	N out
196	LG:132147.3:2000FEB18	1477	1548	forward 1	TM	N out
196	LG:132147.3:2000FEB18	1585	1647	forward 1	TM	N out
196	LG:132147.3:2000FEB18	1690	1752	forward 1	TM	N out
196	LG:132147.3:2000FEB18	2560	2637	forward 1	TM	N out

196	LG:132147.3:2000FEB18	2731	2790	forward 1	TM	N out
196	LG:132147.3:2000FEB18	2908	2976	forward 1	TM	N out
196	LG:132147.3:2000FEB18	3082	3168	forward 1	TM	N out
196	LG:132147.3:2000FEB18	3184	3243	forward 1	TM	N out
196	LG:132147.3:2000FEB18	3376	3462	forward 1	TM	N out
196	LG:132147.3:2000FEB18	1451	1531	forward 2	TM	N out
196	LG:132147.3:2000FEB18	1538	1615	forward 2	TM	N out
196	LG:132147.3:2000FEB18	2741	2827	forward 2	TM	N out
196	LG:132147.3:2000FEB18	2960	3031	forward 2	TM	N out
196	LG:132147.3:2000FEB18	3050	3112	forward 2	TM	N out
196	LG:132147.3:2000FEB18	1626	1703	forward 3	TM	N in
196	LG:132147.3:2000FEB18	2508	2594	forward 3	TM	N in
196	LG:132147.3:2000FEB18	2919	2987	forward 3	TM	N in
196	LG:132147.3:2000FEB18	3177	3263	forward 3	TM	N in
196	LG:132147.3:2000FEB18	3372	3422	forward 3	TM	N in
197	LI:036034.1:2000FEB01	157	219	forward 1	TM	N out
197	LI:036034.1:2000FEB01	395	457	forward 2	TM	N in
197	LI:036034.1:2000FEB01	479	541	forward 2	TM	N in
197	LI:036034.1:2000FEB01	563	625	forward 2	TM	N in
197	LI:036034.1:2000FEB01	647	709	forward 2	TM	N in
198	LG:162161.1:2000MAY19	372	458	forward 3	TM	N in
199	LG:407214.10:2000MAY19	34	120	forward 1	TM	N out
199	LG:407214.10:2000MAY19	44	124	forward 2	TM	N out
199	LG:407214.10:2000MAY19	203	289	forward 2	TM	N out
200	LG:204626.1:2000MAY19	19	99	forward 1	TM	N out
202	LI:476342.1:2000MAY01	39	122	forward 3	TM	N out
203	LI:1072759.1:2000MAY01	409	495	forward 1	TM	N in
203	LI:1072759.1:2000MAY01	889	951	forward 1	TM	N in
203	LI:1072759.1:2000MAY01	1387	1458	forward 1	TM	N in
203	LI:1072759.1:2000MAY01	1687	1770	forward 1	TM	N in
203	LI:1072759.1:2000MAY01	392	478	forward 2	TM	N out
203	LI:1072759.1:2000MAY01	1055	1132	forward 2	TM	N out
203	LI:1072759.1:2000MAY01	1424	1507	forward 2	TM	N out
203	LI:1072759.1:2000MAY01	1694	1768	forward 2	TM	N out
203	LI:1072759.1:2000MAY01	1191	1277	forward 3	TM	N out
203	LI:1072759.1:2000MAY01	1677	1760	forward 3	TM	N out
204	LG:998857.1:2000FEB18	1195	1281	forward 1	TM	N in
204	LG:998857.1:2000FEB18	164	226	forward 2	TM	N out
204	LG:998857.1:2000FEB18	344	400	forward 2	TM	N out
204	LG:998857.1:2000FEB18	398	460	forward 2	TM	N out
204	LG:998857.1:2000FEB18	1478	1561	forward 2	TM	N out
205	LG:482261.1:2000FEB18	19	93	forward 1	TM	N out
205	LG:482261.1:2000FEB18	890	961	forward 2	TM	N out
205	LG:482261.1:2000FEB18	1070	1123	forward 2	TM	N out
205	LG:482261.1:2000FEB18	21	89	forward 3	TM	N out
205	LG:482261.1:2000FEB18	1242	1292	forward 3	TM	N out
206	LG:480328.1:2000FEB18	436	522	forward 1	TM	N out
206	LG:480328.1:2000FEB18	568	642	forward 1	TM	N out
206	LG:480328.1:2000FEB18	769	849	forward 1	TM	N out
206	LG:480328.1:2000FEB18	967	1029	forward 1	TM	N out
206	LG:480328.1:2000FEB18	56	130	forward 2	TM	N in
206	LG:480328.1:2000FEB18	194	280	forward 2	TM	N in
206	LG:480328.1:2000FEB18	396	482	forward 3	TM	N out
206	LG:480328.1:2000FEB18	747	818	forward 3	TM	N out
207	LG:311197.1:2000MAY19	241	315	forward 1	TM	N in
207	LG:311197.1:2000MAY19	527	613	forward 2	TM	N out

WO 01/62927

PCT/US01/06059

208	LG:1054883.1:2000MAY19	76	129	forward 1	TM	N out
208	LG:1054883.1:2000MAY19	83	145	forward 2	TM	N out
209	LG:399395.1:2000MAY19	163	216	forward 1	TM	N out
211	LI:272913.22:2000MAY01	37	123	forward 1	TM	N in

TABLE 4

SEQ ID	Component ID	Start	Stop	SEQ ID	Component ID	Start	Stop	SEQ ID	Component ID	Start	Stop
1	g1260446	2	316	4	70513533D1	496	1069	5	60123152B1	714	1379
1	6791379H1	1	397	4	70317606D1	522	961	5	1602161H1	603	798
1	g1614819	215	655	4	1296898H1	262	495	5	6217784H1	857	1351
1	g1647514	244	543	4	6338333H1	640	1159	5	6217935H1	859	1356
2	5911492F8	1	467	4	g4332126	1	454	5	1473544R6	214	653
2	5911492H1	1	271	4	2659966F6	1	192	5	1473544T1	249	653
2	5911492T8	303	633	4	2659966T6	1	194	5	1473544H1	408	653
3	5311056H1	591	753	4	70513545V1	31	585	5	60123139B1	948	1362
3	6866213H1	784	1388	4	70514030D1	31	577	5	60124858B2	982	1362
3	5659105H1	1261	1340	4	70514030V1	31	589	5	g4457589	222	601
3	5498383R6	1291	1674	4	70513041V1	31	366	5	1473544T6	247	569
3	g5553287	1	315	4	2659966H1	1	247	5	g715576	191	567
3	6989857H1	1	436	4	70512591V1	31	171	5	g993694	255	560
3	6955370H1	22	540	4	70512591D1	31	169	5	2866203H1	459	557
3	g4534562	24	504	4	70318172D1	57	447	5	g682774	196	472
3	g4390046	24	500	4	70513426V1	61	680	5	g3213293	1	454
3	g1192915	25	170	4	70320498D1	247	638	5	g3148379	141	356
3	g2003054	31	344	4	034207H1	1	290	6	4835189H1	679	932
3	6818987J1	33	250	4	70515532V1	24	612	6	g1920265	798	1061
3	6818431J1	33	570	4	70317785D1	295	738	6	4541025H1	804	1057
3	g2003419	45	421	4	70516168V1	24	391	6	2330975H1	952	1172
3	g1551472	61	213	4	70321122D1	370	783	6	2330975R6	952	1311
3	6147606H1	71	625	4	70514482D1	24	278	6	2430651T6	1141	1667
3	6990907H1	383	885	4	70514482V1	24	278	6	2571536T6	1144	1680
3	6866026H1	381	973	4	70318245D1	25	395	6	3878850T6	1244	1662
3	7067123H1	525	1069	4	70318040D1	29	409	6	g2930491	1302	1714
3	5498383F6	573	1055	4	70514959V1	1	300	6	g3844521	1455	1711
3	5498383H1	573	811	5	g2958900	175	294	6	6623593H1	104	689
4	1709025H1	198	445	5	167606H1	1	173	6	6623593J1	634	1236
4	70319971D1	397	784	5	60123139D2	1	307	6	4724029H1	1	133
4	70320592D1	412	733	5	60123152D1	1	368	6	5577111H1	14	200
4	70320769D1	462	809	5	60123139D1	11	156	6	g2183982	84	445
4	70317687D1	464	962	5	6127857H1	1	475	6	3878850F6	119	524
4	6483492H1	184	713	5	g706800	1	340	6	3878850H1	120	399
4	5006925H1	88	362	5	g690740	11	385	6	g2783388	239	721
				5	4454268H1	377	645	6	g2785249	239	710

6	g1920471	284	439	8	2346879T6	167	442	8	3881903H1	1460	1700
6	g1784782	388	828	8	2313878H1	229	495	8	3014303T6	1504	1714
6	6551102H1	429	951	8	6081177H1	264	720	8	490059H1	1515	1763
6	6550902H1	429	1042	8	3773491H1	306	476	8	1803962T6	1575	1714
6	6551002H1	429	957	8	6413047H1	401	935	8	692274H1	1583	1765
6	g3645387	447	909	8	4212712H1	407	675	8	2313878T6	1587	1714
6	2430651H1	448	703	8	6918705H1	413	837	8	g2436491	1623	1714
6	2430651R6	448	680	8	3014942H1	416	684	8	g3277091	1654	1714
6	3712318H1	471	759	8	1807684F6	483	905	8	3319247H1	1718	1969
6	g981405	485	787	8	1803962F6	483	841	8	4917483H1	1916	2145
6	g1975879	1	219	8	1803962H1	483	760	8	6826179J1	1971	2309
6	g1784993	585	909	8	1807684H1	483	746	8	6826179H1	1971	2309
6	1467511T7	1	438	8	3876768H1	529	819	9	3088820H1	537	807
6	6326238H1	8	306	8	6024789H1	553	695	9	2012535H1	561	787
6	526662H1	25	208	8	4062033H1	555	779	9	3232447H1	618	863
6	2856544H1	34	146	8	4634711H1	615	877	9	2536817H2	670	926
7	1980062R6	2	491	8	3014303H1	667	964	9	g2209764	832	1178
7	1980059H1	1	280	8	3014303F6	667	1096	9	7260050H1	1	537
7	5573392H1	30	283	8	1633402H1	672	885	9	2457623F6	101	601
8	2642346H1	1	239	8	5865873H1	725	997	9	6987326H1	110	654
8	2662640H1	2	95	8	6552378H1	881	1430	9	7032756H1	151	720
8	2523793H1	11	212	8	2892794H1	907	1089	9	6457158H1	350	878
8	3158877H1	12	278	8	2892794F6	907	1389	9	2890632F6	351	531
8	3539049H1	13	237	8	6406936H1	935	1476	9	2893095F6	351	909
8	3179390H1	13	320	8	4902732H1	962	1225	9	2893095H1	351	614
8	5543678H1	15	210	8	1492959H1	970	1171	9	7166124H1	401	930
8	6834974H1	15	623	8	g1686447	974	1299	9	4760777H1	427	714
8	257055R6	16	423	8	5929258H1	974	1266	9	3160745H1	437	710
8	133343H1	16	157	8	3509940H1	974	1230	9	g2006716	479	776
8	484198H1	16	163	8	4588644H1	983	1230	9	g2209659	481	940
8	257055H1	17	224	8	6979634H1	991	1412	9	3362481H1	514	763
8	2346879H1	18	247	8	946914H1	1010	1310	9	1751755H1	520	735
8	2346879F6	18	442	8	g5854943	1083	1460	9	6141843H1	536	793
8	g1985882	19	366	8	5544986H1	1095	1303	10	6584158H1	14	592
8	3156918H1	21	155	8	3860122H1	1133	1400	10	3401851H1	4	241
8	4934879H1	22	297	8	g4260489	1228	1701	10	3403550H1	1	259
8	g1751206	41	388	8	1724521H1	1385	1493	10	3404251H1	3	258
8	4024568H1	42	189	8	1724521F6	1385	1714	10	g1137215	2430	2639
8	258140H1	58	433	8	3026692H1	1429	1687	10	3378612H1	2451	2638

10	6584049H1	6	562	12	70557043V1	221	595	14	6420632H1	79	637
10	3402034H1	10	229	12	70562198V1	227	819	14	2195346F6	1	450
10	9307503	16	2638	12	705623312V1	245	894	14	2195346H1	344	450
10	70779637V1	2077	2632	12	70561005V1	249	895	14	3933140F6	11	351
10	7258175H1	2121	2603	12	70559925V1	254	840	14	3933140H1	96	351
10	3961980H1	2130	2252	12	70556220V1	274	841	14	6020638H1	1	263
10	6584189T1	2152	2553	12	70561793V1	282	939	14	95880310	52	255
10	6584158T1	2159	2659	12	70559002V1	293	888	14	92156573	11	254
10	93785069	2394	2641	12	6127888H1	351	824	14	92264198	30	253
10	981592T6	2170	2701	12	70557039V1	359	611	14	6936789H1	11	173
10	3385383T6	2171	2601	12	70559636V1	391	1033	14	5043956H1	11	159
10	1221889T6	2202	2700	12	70561656V1	409	852	15	1897573H1	1	259
10	70779908V1	2348	2654	12	70560742V1	507	990	15	4337619T6	137	645
10	70776665V1	1884	2562	12	70561641V1	594	1066	15	5436383H1	598	849
10	70775642V1	1981	2519	12	9873095	657	1032	16	1671029F6	1	443
10	70776673V1	2022	2640	12	70449484V1	674	894	16	1671029H1	72	144
10	6584049T1	2035	2569	12	6848717H1	732	1253	16	1671029T6	388	443
10	3401765H1	2050	2287	12	70558944V1	1495	2173	16	92238932	388	443
10	3401379H1	15	260	12	70450051V1	1550	1731	16	92243675	388	443
10	3404170H1	18	267	12	70561063V1	1607	2025	17	92219785	236	531
11	6905943H1	1	534	12	95361507	1719	2172	17	5099781H1	378	604
12	70562343V1	1	628	12	94735148	1778	2172	17	3254347R6	527	1132
12	70560334V1	1	538	12	94875543	1779	2195	17	5668261H1	661	889
12	70559056V1	2	275	12	758040T6	1784	2147	17	4289028H1	781	1061
12	70560532V1	12	269	12	9872996	1824	2188	17	93931955	822	1267
12	70559641V1	12	383	12	93840560	1830	2175	17	93401348	823	1271
12	70559213V1	12	511	12	94333943	2114	2199	17	92825326	825	1144
12	70561550V1	12	575	13	2734453F6	1	435	17	2750359R6	824	1242
12	70561738V1	12	582	13	2734453H1	1	252	17	2750359T6	847	1241
12	70559422V1	13	631	14	4860612T7	1184	1574	17	2351445H1	151	367
12	70559480V1	12	687	14	9902319	1020	1492	17	2750359H1	968	1241
12	758040H1	12	272	14	9778351	1137	1386	17	2203345H1	1018	1169
12	70559415V1	13	605	14	91638619	1140	1268	17	3254347H1	979	1089
12	758040R6	12	310	14	6929341H1	724	1222	17	4822216H1	1202	1393
12	70561876V1	13	585	14	1675892T6	754	1203	17	3184753F6	1	472
12	70559130V1	35	384	14	1720010F6	696	1079	17	5108214T6	60	258
12	92576304	119	2175	14	1720010H1	696	922	17	3184753T6	60	312
12	70561182V1	150	812	14	4711951H1	675	833	17	94287070	152	617
12	70560479V1	217	834	14	5496406H1	546	795	17	92524411	160	493

17	3184753H1	228	472	20	3456613H1	177	427	21	2670050H1	722	974
17	g2788109	236	513	20	4923878H1	211	494	21	4118973H1	762	1013
18	6829315H1	314	884	20	6847871H1	274	451	21	2744119H1	784	968
18	g3109791	492	811	20	g5151844	373	585	21	g1081212	787	1107
18	g5452473	492	650	21	g4281322	2017	2463	21	5576610H1	879	1134
18	g4564783	26	423	21	3934096H1	2040	2211	21	6418368H1	926	1226
18	g5438746	1	423	21	3934274H1	2042	2187	21	5864111H1	950	1233
18	g3805312	34	423	21	1648485T6	2045	2159	21	5033538H1	997	1264
18	g4372490	78	423	21	1648242H1	2052	2198	21	35111167H1	1010	1288
18	g2954208	76	423	21	1648485H1	2052	2198	21	5805062H1	1021	1225
18	g2954218	142	422	21	1648485F6	2052	2198	21	4873652H1	1038	1293
18	g3307490	142	344	21	4676772H1	2056	2317	21	6614566H1	1064	1565
18	2011384H1	190	263	21	1004843T6	2092	2693	21	2645969F6	1085	1588
19	3106785H1	747	1023	21	g4533314	2101	2207	21	2645969H1	1085	1330
19	g565430	783	1150	21	3416826H1	2134	2365	21	g2112831	1112	1336
19	g1505888	812	1132	21	5304471H1	2184	2431	21	3742279H1	1144	1422
19	g892901	859	1159	21	1310089F6	2250	2718	21	6334985H1	1249	1754
19	g892900	1	179	21	1310089H1	2250	2448	21	5292787H2	1287	1357
19	3436153H1	1	269	21	2461918H1	2266	2461	21	g853299	1363	1679
19	g4737696	1	452	21	g4330296	2658	2953	21	5742830H1	1399	1713
19	839090H1	73	301	21	g3178479	2713	2950	21	777713H1	1400	1625
19	g938671H1	206	685	21	g856243	2748	3020	21	777713R6	1401	1702
19	g939071H1	207	731	21	3951063F6	1	417	21	2291319H1	1424	1651
19	g646973	427	675	21	3951063H1	1	285	21	3241291H1	1436	1673
19	g1277657	484	934	21	g2114724	215	609	21	1832630H1	1481	1750
19	2755039H1	596	767	21	1804812F6	311	871	21	1804812T6	1571	2172
19	838332T6	615	1116	21	1804812H1	311	582	21	1481768H1	1572	1793
19	4800144H1	651	916	21	1804772H1	311	530	21	3730896H1	1583	1874
19	2084151H1	664	945	21	2662164H1	346	588	21	4442950H1	1631	1773
20	6199407H1	1	558	21	2011927H1	365	608	21	2794894H1	1648	1896
20	2519336F6	37	448	21	4863613H1	380	645	21	4161422H1	1664	1913
20	2519336H1	37	198	21	3934325H1	386	672	21	4163675H1	1665	1779
20	6793417H1	61	331	21	3763239H1	402	671	21	g2787436	1672	1875
20	1639314H1	71	248	21	1004843R6	502	864	21	1898917T6	1693	2159
20	6818283H1	75	652	21	1004843H1	502	706	21	5026603H1	1695	1945
20	1984661R6	94	490	21	g846375	547	889	21	3726809H1	1753	2047
20	1984662H1	94	368	21	5810205H1	585	885	21	1891483T6	1803	2160
20	1984661T6	127	543	21	384851H1	652	926	21	2293309H1	1836	2077
20	4021069H1	177	388	21	g1861137	688	1180	21	1214225H1	1850	2020

21	g856337	1855	2128	22	5681165H1	629	889	22	g3147585	1357	1758
21	g3733909	1913	2198	22	5277977H1	637	892	22	g2053942	1361	1759
21	g3890798	1917	2386	22	4645753H1	659	921	22	g1332315	1371	1839
21	5696574H1	1925	2048	22	6193452H1	705	1000	22	3918780H1	1373	1665
21	g1081162	1942	2201	22	6193441H1	705	963	22	g3884754	1379	1765
21	2645969T6	1964	2222	22	746066H1	713	961	22	5207656H1	1382	1616
21	g1860963	1970	2387	22	4771093H1	754	1022	22	2292948H1	1388	1552
21	777713T6	1983	2420	22	g2054100	771	1123	22	1373715H1	1388	1603
21	g4149122	1984	2203	22	g4762720	796	1252	22	4742391H1	1388	1647
21	4875545H1	1994	2292	22	g2321843	801	1217	22	2749392H1	1400	1627
22	487652H1	1	242	22	g3017276	804	1256	22	g1241849	1408	1559
22	487652R6	1	232	22	g483457	850	1222	22	g6046888	1408	1785
22	g2003010	47	328	22	g4762705	865	1252	22	2294209T6	1428	1945
22	6442754H1	49	581	22	4338534H1	870	1155	22	g1471742	1441	1766
22	6152037H1	50	234	22	628211H1	887	1051	22	g4332659	1471	1772
22	7072795H1	75	624	22	5563275H1	922	1144	22	g3842415	1473	1785
22	3126080H1	75	234	22	5286207H1	952	1210	22	g2841585	1474	1756
22	491193H1	83	234	22	6160626H1	953	1244	22	g2003011	1479	1772
22	4751976H1	90	232	22	6838871H1	1013	1491	22	2501320H1	1513	1738
22	7100707H1	100	609	22	2400686H1	1068	1153	22	g3961438	1525	1988
22	2292643H1	102	232	22	g866503	1107	1440	22	g2022791	1559	1757
22	6140970H1	105	232	22	6747627H1	1122	1593	22	g6132921	1559	1977
22	4066069H1	110	234	22	g920433	1143	1481	22	5690247H1	1568	1843
22	5007390H1	113	231	22	2294209R6	1179	1688	22	g1496515	1580	1977
22	4773161H1	130	415	22	2294209H1	1179	1444	22	g2252260	1620	1984
22	6993149H1	145	680	22	g1471741	1213	1600	22	g3739177	1650	1831
22	572575H1	150	232	22	g1332314	1213	1712	22	5338636H1	1671	1915
22	5756677H1	180	483	22	6021757H1	1214	1822	22	5710854H2	1686	2004
22	6064882H1	346	644	22	2189963H1	1229	1505	22	3223930H1	1686	1772
22	5372689H1	367	514	22	g5766946	1310	1760	22	4255692H1	1701	1756
22	3123257H1	367	493	22	g2524669	1309	1776	22	g1210253	1725	1980
22	g2022792	381	648	22	g2178309	1317	1448	22	g920434	1730	1946
22	5068149H1	503	782	22	g4762067	1326	1779	22	g1210208	1763	1980
22	5894157H1	560	759	22	g5361693	1328	1778	22	4320678H1	1841	1973
22	5898812H1	560	853	22	g4074013	1332	1773	22	g884068	1924	2233
22	2621801H1	561	824	22	g5849550	1331	1762	23	6991066H1	1	190
22	6401738H1	574	844	22	6736883H1	1355	1730	23	4753733H1	121	279
22	2122453H1	622	902	22	g2183535	1356	1766	23	4753733F8	122	613
22	2122453F6	622	764	22	g4332913	1357	1772	23	7065882H1	183	679

23	95676063	251	722	25	6862186H1	519	806	27	2966432H1	1421	1627
23	1576986F6	270	661	25	95543132	541	939	27	93419012	1500	1961
23	1576986H1	270	349	25	94196555	541	821	27	92178116	1505	1630
23	1576986T6	275	683	25	95636120	541	982	27	92715860	1512	1962
23	93843718	364	724	25	93246528	541	923	27	3891858T6	1559	1901
23	93750491	408	722	25	95636142	541	1018	27	3891858F6	1566	1961
23	3604635H1	409	584	25	92568732	577	1024	27	3891858H1	1566	1796
23	9793096	600	881	25	3747228H1	589	887	27	925628T6	1574	1943
24	6756043H1	101	504	25	6600395H1	587	999	27	925628H1	1574	1797
24	3845553H1	368	574	25	6978931H1	605	954	27	95113339	1766	2184
24	5057288F9	382	1021	25	2240520H1	629	730	27	7286802H1	1	500
24	5057288H1	382	660	25	2240520F6	641	967	27	3490274H1	26	319
24	5065461F8	404	839	25	5626829H1	663	931	27	6817649H1	55	308
24	2359950H1	625	748	25	92162199	663	863	27	6779822H1	68	617
24	6112349H1	792	1081	25	6862047H1	699	978	27	1343838H1	166	433
24	5444375H1	912	1131	25	7281807H1	809	1041	28	6827746J1	1	328
24	94264503	989	1390	25	92209650	815	1308	28	94372436	2	487
24	3385663T6	1011	1248	25	6216076H1	841	1322	28	7277307H1	1	320
24	3845553T6	1009	1248	25	7238641H1	862	978	28	91109781	68	2457
24	3385663F6	1	315	25	6210267H1	868	970	28	92987914	238	414
24	3385663H1	1	257	25	7039186H1	940	1508	28	3242379H1	460	594
24	5468404H1	3	271	25	92209760	1092	1569	28	5843682H1	675	814
24	5468404F8	3	587	26	7281748H1	1	439	28	6244385H1	750	960
24	5072501H1	11	292	26	7281705H1	1	574	28	3560794H1	878	978
24	6917202H1	10	259	27	925628R6	1574	1961	28	6146228H1	1389	1559
24	4148204T6	1176	1391	27	6466550H1	228	746	28	95454470	1759	2219
24	4148204H1	1183	1435	27	035057H1	483	638	28	95634013	1818	2245
24	4148204F6	1183	1390	27	2173285F6	571	968	28	93933775	1835	2188
24	94532148	1256	1361	27	2173285H1	571	762	28	6824121H1	1864	2239
24	213841R6	1325	1390	27	1743077R6	577	1069	28	6824121J1	1864	2239
24	213841T6	1325	1393	27	1743077H1	577	892	28	6789580H1	1898	2361
24	213841H1	1325	1390	27	3344276H1	598	856	28	95596185	1915	2221
24	213833H1	1325	1390	27	6579246H1	697	1025	28	91137662	1994	2365
25	7204172H1	1	536	27	5637939F8	825	1101	28	93917233	2020	2409
25	6819816H1	368	566	27	5637939H1	825	1082	28	3222719H1	2019	2299
25	6836688H1	383	960	27	5624530R8	836	1190	28	94487317	2029	2459
25	6349781H2	434	616	27	5637939R8	1016	1380	28	92878126	2045	2386
25	7280863H1	477	880	27	3483518H1	1163	1470	28	925319R6	2061	2458
25	94081510	513	860	27	6817649J1	1402	1969	28	925319T6	2061	2419

28	925319H1	2061	2370	29	70254664V1	444	930	29	70248162V1	1486	1604
28	g6463796	2103	2467	29	70254648V1	463	958	29	70254647V1	612	1111
28	2175458H1	2139	2397	29	70257273V1	531	636	29	7077379H1	627	1163
28	g1153903	2165	2458	29	g3778732	559	1002	29	7237267H1	1907	2197
28	g1114183	2165	2444	29	4023624H1	1	286	29	70660270V1	1926	2390
28	g3844190	2203	2458	29	70249613V1	1	198	29	70255193V1	1976	2586
28	6193937H1	2265	2534	29	3269702H1	8	254	29	70656301V1	1979	2231
28	g5364673	2276	2476	29	70249634V1	142	374	29	71273576V1	1979	2197
29	6450124H1	992	1374	29	1286494H1	165	402	29	6620779H1	2006	2603
29	70254601V1	1036	1539	29	7191139H2	175	755	29	70655675V1	2023	2430
29	70250522V1	1050	1299	29	5841654H1	175	446	29	70255204V1	2038	2632
29	70255407V1	1073	1570	29	7120789H1	398	702	29	1741694T6	2052	2625
29	g2004277	1088	1409	29	70255670V1	413	949	29	6121206F8	2130	2669
29	70247748V1	1116	1378	29	70255767V1	1672	2119	29	5267406H1	2070	2344
29	70249044V1	1117	1269	29	3272240F6	1165	1617	29	6120592H1	2110	2682
29	70256246V1	1130	1541	29	3272240H1	1166	1413	29	6128080H1	2110	2689
29	70248757V1	1132	1311	29	70257558V1	1171	1601	29	6120292H1	2110	2671
29	7331424H1	1149	1638	29	70248599V1	1202	1420	29	6127177H1	2110	2671
29	70248822V1	1157	1333	29	70250795V1	1247	1524	29	3269702T6	2111	2623
29	70656623V1	1723	2219	29	70249059V1	1304	1679	29	70254909V1	2152	2688
29	661089H1	1723	1986	29	70250085V1	1310	1485	29	4001246R6	2158	2517
29	661089R6	1723	2224	29	70251290V1	1314	1573	29	4001246H1	2158	2458
29	6444789H1	1742	2329	29	70255060V1	1326	1720	29	70659041V1	2163	2394
29	70248827V1	1769	2103	29	70254762V1	1330	1860	29	g5864796	2171	2685
29	6551207H1	1771	2379	29	70257824V1	1339	1525	29	g2913171	2184	2622
29	6745795H1	1775	1933	29	70255199V1	1341	1753	29	6752303H1	2193	2671
29	6977014H1	1787	2347	29	70250692V1	1345	1576	29	70658326V1	2194	2559
29	70258294V1	1791	2007	29	70257468V1	1347	1462	29	70256172V1	2209	2671
29	70254633V1	1805	2279	29	70251556V1	1351	1435	29	2287809R6	2232	2681
29	70255157V1	1838	2268	29	70255729V1	1361	1869	29	2287809T6	2232	2629
29	6746363H1	1854	2506	29	70255867V1	1392	1903	29	2287809H1	2232	2458
29	70655542V1	1865	1948	29	70255422V1	1366	1870	29	6834934H1	2233	2671
29	6561163H1	1872	2335	29	70254725V1	1690	2218	29	2255790H1	2260	2543
29	7239061H1	1879	2417	29	70254417V1	1671	1916	29	70657495V1	2264	2391
29	70247905V1	1879	2117	29	7033280H1	1691	2365	29	6835034H1	2288	2570
29	70255827V1	764	1239	29	1741694R6	1410	1866	29	70254456V1	2316	2671
29	70255250V1	842	1401	29	1741694H1	1410	1587	29	661089T6	2355	2639
29	70248754V1	866	1233	29	70256209V1	1414	1915	29	70249168V1	2452	2682
29	70256314V1	917	1512	29	70792331H1	1418	1892	29	6059187H1	2608	2671

29	6059196H1	2606	2682	30	3385663T6	1033	1284	30	6917202H1	10	263
29	70255727V1	977	1493	30	3845553T6	1031	1284	30	3385663H1	1	261
29	70257455V1	1692	1951	30	70776533V1	1086	1284	30	5468404F8	3	596
29	70257816V1	1397	1604	30	70776877V1	1087	1284	30	3385663F6	1	319
29	70257088V2	1659	1980	30	4148204T6	1174	1389	30	70775648V1	4	416
29	70255058V1	1663	2172	30	4148204F6	1181	1388	30	70778292V1	4	577
29	5779331H1	1663	1928	30	4148204H1	1181	1433	30	70779496V1	4	568
29	70248161V1	1487	1604	30	94532148	1254	1359	30	70775549V1	4	538
29	6536779H1	1490	2108	30	5072501H1	11	296	30	70776773V1	4	576
29	70254435V1	1509	2113	30	70770035V1	207	641	31	93254719	1	445
29	70254449V1	1538	1906	30	70769523V1	245	581	31	95745388	1	462
29	70257853V1	1570	1900	30	70779544V1	337	548	31	93959110	1	353
29	70254760V1	1571	1970	30	70776851V1	339	867	31	9897505	1	215
29	70255434V1	1577	2080	30	70779695V1	4	418	31	95233046	1	295
29	5191169H1	1609	1875	30	5468404H1	3	275	31	92929868	1	406
29	70257088V1	1659	1980	30	6756043H1	102	509	31	93016800	1	358
29	70255260V1	1694	2245	30	7190732H1	163	722	31	6202689H1	1	548
29	70255174V1	1695	2113	30	71087882V1	4	560	31	92435161	9	456
29	9756281	1698	2066	30	70777729V1	4	492	31	4707801H1	1197	1296
29	70247381V1	1704	2145	30	70781241V1	4	467	31	806768H1	1211	1421
29	70659718V1	1723	2028	30	70780840V1	4	442	31	6915008H1	1320	1761
29	70254418V1	675	1188	30	70779497V1	342	547	31	3036834H1	1578	1846
29	70258031V1	699	905	30	3845553H1	372	583	31	3036834F6	1578	2016
29	7178470H1	651	1108	30	5057288F9	386	1043	31	5387714H1	1632	1886
29	70256308V1	672	1122	30	5057288H1	386	669	31	7122625H1	443	835
30	70780965V1	4	464	30	70776801V1	399	933	31	6915116J1	622	1129
30	2359950H1	634	758	30	5065461F8	408	858	31	5092947H1	899	1177
30	70777278V1	648	1196	30	70778496V1	429	737	31	5370334H1	939	1050
30	70780989V1	653	1111	30	70777098V1	456	752	31	5092947F6	899	1330
30	70779952V1	697	1217	30	70780976V1	476	1081	31	5017752H1	959	1229
30	70777355V1	715	1195	30	70777266V1	503	1128	31	4707801F6	1197	1714
30	70771142V1	716	1207	30	70776726V1	513	1093	31	5505129H1	17	282
30	70775784V1	751	1132	30	70772943V1	541	740	31	5514010H1	17	254
30	70769785V1	764	1127	30	70777725V1	576	1166	31	5505122H1	17	228
30	6112349H1	809	1108	30	70779028V1	613	766	31	1225071H1	52	262
30	70776223V1	839	1239	30	213833H1	1323	1388	31	92929769	56	178
30	5444375H1	933	1164	30	213841T6	1323	1391	31	6314660H1	60	613
30	5057288T8	997	1251	30	213841R6	1323	1388	31	2682720F6	173	606
30	94264503	1011	1388	30	213841H1	1323	1388	31	6516656H1	275	736

31	6201718H1	312	599	32	042027H1	310	561	36	6769153J1	1	529
31	2682720H1	324	606	32	g1291521	316	662	36	6764824J1	3	589
32	3665294H1	706	823	32	030668H1	319	524	36	g6048166	3	423
32	g1139437	903	977	32	g897225	340	705	37	653157H1	82	310
32	3663680H1	706	950	32	g921054	340	660	37	509350H1	107	307
32	5040414H1	731	947	32	g897232	340	676	37	3386816H1	1	183
32	g1139431	904	977	32	5018641T6	368	996	37	3888124H1	4	268
32	1772915R6	911	1308	32	g2933440	374	696	37	6779840H1	9	549
32	1772915H1	911	966	32	1876772T6	379	940	37	3159561H1	58	220
32	4849209H1	1000	1259	32	g2703395	564	979	37	6250084H1	67	593
32	1772915T6	1024	1540	32	2652012T6	566	930	37	2809974H1	67	324
32	g4691031	1318	1588	32	g1858336	575	1000	37	3386816T6	1	622
32	6298418H1	1	314	32	3124683H1	622	881	37	3672749H1	81	364
32	3403590H1	1	232	32	g1313829	631	867	37	4005442H1	2	285
32	6327716H1	98	415	32	4200739H1	671	956	37	70821167V1	627	1066
32	g1068995	238	588	32	2244549H1	671	882	37	70822737V1	643	949
32	6921019H1	255	457	32	2684460H1	696	909	37	70832705V1	715	823
32	4307678H1	254	563	33	g3232386	1	362	37	412478T6	835	1013
32	g1858272	267	664	33	g3181700	7	419	37	6779840J1	894	1315
32	2812311H1	503	816	33	6109493H1	322	613	37	g2631213	986	1049
32	2812294H1	505	826	34	3081155F6	1	417	37	5501816H1	1053	1261
32	4976432H1	536	792	34	3081155H1	2	315	37	5501816T6	1053	1293
32	g5037963	518	976	34	3081155T6	31	463	37	4005442T6	487	1008
32	4976496H1	536	801	34	g1648354	58	455	37	70818940V1	506	1068
32	g3736768	525	978	34	5800009H1	103	522	37	059074H1	601	765
32	g4986041	540	979	35	3517533R6	332	653	37	28622291H1	375	652
32	4124067H1	552	792	35	3517533H1	332	621	37	g3934153	376	841
32	g4073888	527	978	35	g5178468	190	667	37	g3900180	404	840
32	g5526438	564	978	35	3504115H1	162	477	37	391612F1	432	1051
32	5209523H1	413	705	35	3082646H1	123	415	37	g895347	1	84
32	4109819H1	416	696	35	259700H1	1	278	37	4005442F6	1	326
32	3726063H1	426	688	35	7169509H1	1	477	37	391612R1	353	898
32	4671035H1	421	693	35	6768733H1	58	292	37	412478R6	373	699
32	2311003H1	454	685	35	3176620H1	66	320	37	412478H1	373	600
32	5206768H1	478	723	35	3176620F6	66	551	37	3386816T6	337	818
32	679406H1	480	737	35	4613367H1	72	326	37	1951349T6	296	802
32	829499H1	273	519	36	6753724J1	1	550	37	6947424H1	302	813
32	2655709T6	279	932	36	6769683J1	1	595	38	6980110H1	1	501
32	1494347H1	285	504	36	6772986J1	3	579	38	g1897936	277	634

38	6264019H1	282	417	41	g1648352	23	428	43	117874R6	1	438
38	309905H1	300	614	41	3023715H1	184	448	43	4229003F6	171	674
38	g2329399	438	689	41	2071327F6	285	584	43	4229003T6	271	738
38	2821248F6	452	766	41	2071327H1	285	475	43	g3895952	404	823
38	2821248H1	452	755	41	6912958J1	351	944	44	g3803941	113	583
38	g954493	494	556	41	4583753H1	378	666	44	g3805589	75	524
38	6291249H1	507	718	41	2071327T6	676	1111	44	g3917967	73	520
38	5636134H1	640	912	41	g4176194	713	1156	44	3817246H1	28	320
38	6758395H1	658	1132	41	g3178308	731	1151	44	2895718H1	1	283
38	1903638H1	701	954	41	g4149782	824	1142	45	4295967F8	1	404
38	4991026H1	847	1075	41	g3797002	837	1307	45	g5811741	166	582
38	4991026F6	847	1335	41	g3797002	974	1088	46	6708077H1	547	1179
38	2821248T6	894	1434	41	g3180193	1135	1214	46	5352502H1	578	664
38	6208066H1	893	1387	42	6937854H1	1927	2414	46	6819988J1	1	605
38	4453533H2	942	1149	42	7032292H1	1837	2263	46	6292367H1	493	609
38	5621950H1	1017	1297	42	7002267H1	1385	1996	46	g4900516	542	664
38	6827666H1	1089	1614	42	7153478H1	1986	2456	47	2607525T6	1	557
38	3165907H1	1298	1569	42	7100968H1	1404	1874	48	5120882F6	1	353
38	4982360H1	1350	1616	42	7035269H1	1372	1791	48	g4072524	256	619
39	5998240H1	1	484	42	7166089H1	1147	1690	49	4936033R6	591	1027
39	458061H1	20	259	42	5637088H1	1339	1516	49	5387945T6	1	633
39	455162H1	20	272	42	6778731H1	949	1515	50	5401502T6	1	364
39	460544R6	20	326	42	2689778H1	1187	1439	51	2205615T6	377	901
39	455966H1	20	255	42	4233860F6	818	1413	51	4150193F6	1	509
39	455162R6	20	521	42	5975002H1	2124	2431	52	g2005559	1	239
39	460544H1	20	245	42	g1423371	869	1290	52	13998332H1	1	227
39	3206542H1	25	202	42	g3424813	874	1290	52	1398471H1	1	238
39	4663964H1	37	289	42	g1859062	1009	1290	52	g2027783	1	329
39	g1807165	81	278	42	1809216F6	809	1271	52	1322674H1	2	254
39	g2401959	129	486	42	1809216H1	1109	1271	52	g2278640	13	442
39	3614680H1	155	272	42	4290553H1	911	1161	52	g2159473	86	551
39	g5101146	180	486	42	6932930H1	733	1101	52	g2162158	123	594
40	6927079H1	1	400	42	g1858731	690	1070	52	g2156015	131	366
40	g922215	84	386	42	4293860H1	818	1065	52	g2156002	208	366
40	g922268	84	327	42	4042230H1	673	849	52	g1954430	228	425
40	g991321	109	483	42	g1423374	236	722	53	2737435F6	1	479
40	4991883T6	268	799	42	6132451H1	275	568	53	2737435T6	398	597
40	1958936H1	672	848	42	6781622H1	1	541	54	4654776T6	126	637
41	6033171H1	1	434	42	5966128H1	192	357	54	3796761F6	1	403

54	3796761H1	1	306	57	g1962414	304	564	59	g3057535	162	618
54	4654776H1	34	285	57	g1376321	264	526	59	6429507H1	305	589
54	4654776F6	34	415	57	g4110945	3	417	59	2865771F6	460	851
54	1729735H1	52	280	57	g4310623	1	416	59	2865771H1	460	746
54	g895564	59	388	57	g2733078	2	384	59	3639153H1	501	824
55	2920075R6	1	427	57	g2835164	3	383	59	g1258980	584	839
55	784499T6	20	470	57	g3241486	1	382	59	3569914F6	601	1111
55	2920075H1	169	427	57	g1376322	2	296	59	3569914H1	601	894
55	269219H1	211	414	57	3351668F6	1	267	59	3569914T6	962	1569
55	269219R1	212	754	57	3351668H1	1	264	59	5327014H1	1048	1291
55	g1978467	356	665	57	1785191H1	10	194	59	1320957H1	1120	1353
56	6183766H1	1	267	57	g2198029	1	129	59	2971828F6	1216	1745
56	g1897936	1	354	57	4050289H1	28	127	59	g3049125	1226	1757
56	3099905H1	19	334	57	4144289H1	20	125	59	3077923H1	1284	1572
56	g2329399	158	409	58	g1979821	1	188	59	g4069747	1301	1758
56	2821248F6	172	486	58	g3835204	1	325	59	g3417824	1307	1758
56	2821248H1	172	475	58	g4072180	19	468	59	5732379H1	1398	1646
56	g954493	214	276	58	3519808H1	81	367	59	5732479H1	1399	1592
56	6291249H1	227	438	58	g843784	232	499	59	2971828H2	1452	1745
56	5636134H1	360	632	58	1614746F6	288	732	59	g1201083	1580	1748
56	1903638H1	421	674	58	1614746H1	288	505	59	4459657H1	1655	1775
56	4991026F6	567	1055	58	1575232H1	365	577	60	60206922U1	296	954
56	4991026H1	567	795	58	g2209653	403	912	60	368741T6	399	902
56	6208066H1	659	1163	58	6322462H1	520	718	60	368741R6	1	397
56	2821248T6	614	1154	58	2892611T6	645	1136	60	368741H1	1	308
56	4453533H2	662	869	58	1614746T6	733	1148	60	g4187853	375	750
56	5621950H1	737	1017	58	g3644249	766	1189	60	368741R1	1	490
56	3165907H1	1018	1289	58	670448R6	800	1186	60	g3838805	409	751
56	4982360H1	1070	1335	58	677184R6	800	1152	61	5963989H1	1	502
56	6417723H1	1325	1610	58	670448T6	800	1149	61	3593809H1	1	298
57	6061626H1	753	1059	58	677184H1	800	1071	61	g1059739	1	137
57	5425067H1	798	1041	58	670448H1	800	1055	61	1850713F6	1	298
57	6331039H1	50	618	58	g2810503	840	1186	61	1850713H1	1	241
57	6132218H1	689	951	58	g2336018	907	1187	62	2872408F6	1	397
57	3499278F6	482	891	58	2892611H1	945	1178	62	2872408H1	367	653
57	3499278H1	645	891	58	1289991H1	1081	1187	62	2872408T6	33	629
57	4657265H1	310	411	59	g3838936	83	488	63	2862618T6	1	569
57	6309037H1	233	724	59	g4085429	126	588	63	2838402H2	1	252
57	6538713H1	284	682	59	g1148294	133	524	63	287364H1	1723	2056

63	2867867H1	198	481	76	2007652T6	225	449	86	60203050V1	304	692
63	2828380H1	522	792	76	2007652R6	225	480	86	70374630D1	304	668
63	2881835T6	606	1167	77	3325402T7	1764	2221	86	60209436U1	1	656
64	2882345T6	1809	2320	78	4654776F6	34	415	86	70373679D1	378	626
64	2820130T6	1421	1994	78	4654776H1	34	285	86	70373094D1	400	786
64	2837371H1	450	548	78	4654776T6	126	637	86	70375597D1	303	605
65	2222227T6	809	1284	79	1476477F6	1	548	86	70373898D1	304	437
66	270908H1	1	102	79	1476477H1	1	205	86	70373514D1	304	437
66	2698411H1	102	402	79	1476477H6	1	207	86	819073H1	1256	1510
66	269967H1	55	354	79	1476485H1	1	85	86	2831185H1	1233	1479
66	2778926H1	24	260	79	1476477T6	120	639	86	70375782D1	380	772
66	2737435H1	1	207	79	1500139H1	86	292	86	70374947D1	304	772
66	2737435F6	19	501	79	1496323H1	591	830	86	9824801	1579	1852
66	2753160H1	1	119	80	5200862H1	1	220	86	70376281D1	472	766
66	2718845H1	1	245	80	5200862F6	1	508	86	60209435U1	102	747
66	2718845F6	1	395	80	5200862T6	371	989	86	92780043	1733	2066
66	2729146H1	107	344	81	450278R6	619	1125	86	2751856R6	1651	2039
66	2690266H1	126	385	81	448904R6	1	390	86	70374895D1	404	947
66	2782984H1	438	571	81	450278R7	619	1046	86	70376064D1	393	947
66	2737435T6	420	617	81	448904H1	1	216	86	70374077D1	472	947
67	2666231T6	1	523	81	446282H1	1	256	86	70373091D1	669	947
68	1729868T6	1414	1975	81	450278H1	1	198	86	70373401D1	782	947
69	3256037T6	1397	1940	81	448904T6	721	1298	86	70376523D1	789	947
70	3394349T6	33	600	81	450278T7	788	1297	86	70375900D1	868	946
71	5387945T6	685	1317	82	807409T6	210	590	86	3999158H1	1844	1992
72	394892F1	793	1443	82	807409R6	210	629	86	70376383D1	606	867
72	402386T6	930	1401	82	807409H1	1	78	86	2751856H1	1651	1923
73	3318809F6	1	438	83	3844814F6	147	721	86	70374800D1	304	837
73	3318809H1	3	276	83	3844814H1	433	721	86	2751062H1	1651	1919
73	3318809T6	227	754	83	3779417H1	1	204	86	70373252D1	300	800
73	3255863H1	438	688	83	3769201H1	63	208	86	70375702D1	340	800
74	3685359H1	1	303	83	3844814T6	63	317	86	60203054V1	353	791
74	3685359F6	42	501	84	1224910R6	1	543	86	70373230D1	359	787
74	3685359T6	1	279	84	1224910H1	881	1024	86	5822141H1	1871	2175
75	3637162H1	1	292	84	1224910T6	98	446	86	6332621H1	626	1098
75	3637162F6	38	490	85	5296190T6	1523	1959	86	1689922F6	1942	2175
75	3699637H1	22	182	86	70375800D1	365	741	86	5820119H1	1871	2166
75	3637162T6	192	740	86	5884784H1	1617	1830	86	94222747	1715	2166
76	2007652H1	225	411	86	60203053V1	286	730	86	93594875	1710	2160

86	g3751911	1700	2160	90	6779728J1	1	370	93	5767313H1	1	246
86	g3847457	1754	2160	90	g3250058	1	408	93	000413H1	1	241
86	g2675135	1863	2160	90	6245903H1	120	532	93	257941H1	1	237
86	g4524280	1891	2160	90	g4333202	140	407	93	2727658H1	1	173
86	g3422353	1857	2153	91	1895443H1	19	272	93	5511002F6	628	905
86	1689922H1	1942	2151	91	4729485H1	86	235	93	g1977359	11	218
86	g1501747	1770	2146	91	5217490H1	1	198	94	894136H1	1	167
86	g2342389	1928	2145	91	1542833H1	1	137	94	893591H1	1	280
86	g846897	1832	2132	91	3836068F6	7	546	94	3685359F6	21	480
86	g4264802	1694	2117	91	1891884H1	19	264	94	3685359H1	21	323
86	g3693061	1788	2117	91	3052342H1	46	335	94	3685359T6	243	521
86	g4269694	1799	2117	91	5735738H1	78	348	95	g1925745	1	143
86	g2269848	1756	2117	91	g5661058	155	347	95	g2752333	16	387
86	g824800	1834	2117	91	3525414H1	171	494	95	g2881366	16	370
86	g3069508	1651	2117	91	3836068H1	7	289	95	3257022H1	28	287
86	g2055928	1783	2117	92	1335071H1	515	737	95	2380565F6	186	681
86	g2539104	1673	2117	92	1353732H1	535	775	95	2380565H1	186	401
86	g2901266	2005	2113	92	6166536H1	736	1266	95	6354028H1	192	471
86	70375731D1	590	1018	92	6483617F9	808	1320	95	2809746H1	248	487
86	g4390710	1751	2184	92	6483617F8	828	1386	95	5110009H1	249	500
87	5059054H1	1	257	92	4326477F6	1	381	95	5110002H1	249	441
87	5059022F9	1	581	92	4326477H1	2	160	95	6354128H1	326	480
87	179544R6	84	529	92	4140846T9	26	550	95	1546477H1	378	584
87	1227952T6	125	595	92	3888314H1	52	314	95	5055767H1	565	845
87	064418H1	286	457	92	7073510H1	119	689	95	3413877H1	572	817
87	179544H1	329	529	92	7081835H1	125	678	95	2360256R6	719	1123
87	g1011391	482	807	92	2079383H1	301	563	95	2360256H1	719	976
87	4667922H1	539	759	92	6405368H1	315	599	95	g1688388	781	1110
88	g4189831	12	63	92	7254119H1	491	935	95	1330559H1	784	1027
88	4721701H1	360	439	92	6420281H1	653	1159	95	g844802	968	1283
88	3123901H1	1	66	92	6759433J1	668	1118	95	g1640803	1007	1243
88	6785315H1	12	481	92	6483617H1	808	1319	96	4895030H1	2484	2784
88	7201126H2	51	570	93	257941R6	1	252	96	g1941651	2486	2951
89	2327449H1	15	248	93	2764170H1	19	259	96	70657184V1	2503	3092
89	2327457T6	1	364	93	7040554H1	149	692	96	g658182	2512	2776
89	2327449T6	1	288	93	6477351H1	541	1039	96	g2718978	2525	2989
89	2327449R6	13	408	93	g3163349	590	1022	96	g715365	2538	2875
89	2327457R6	13	402	93	3209591H1	1	149	96	5298571H1	2539	2819
90	g3694145	26	408	93	5511002H1	628	880	96	4749511H1	2540	2838

96	5298771H1	2539	2804	96	g1780421	3068	3450	96	g3869256	202	4338
96	5298612H1	2540	2664	96	6937801H1	3118	3682	96	2693067H1	559	813
96	6041370H1	2550	3199	96	6500625H1	3113	3727	96	6945783H1	613	1122
96	g1471133	2556	2990	96	g654349	3148	3441	96	2535607H1	838	1099
96	6555238H1	2565	3127	96	g5747781	3156	3445	96	70837439V1	956	1519
96	6556278H1	2565	3092	96	g758975	3157	3485	96	3502278H1	1050	1359
96	2913641H1	2604	2878	96	g564984	3156	3445	96	g1779649	1215	1673
96	5825362H1	2609	3122	96	g1357788	3163	3465	96	60209482U1	1264	1763
96	6400248H1	2624	2806	96	g2931035	3178	3445	96	495759R1	1304	1782
96	g1719353	2634	3091	96	155875T6	3180	3849	96	495759R6	1305	1682
96	g1960091	2671	3185	96	3874011H1	3185	3455	96	495759H1	1305	1559
96	6829782H1	2683	3131	96	g1190099	3218	3440	96	70375665D1	1438	2033
96	6829782J1	2683	3131	96	3117949H1	3251	3435	96	60207458U1	1487	2000
96	g6139947	2710	2989	96	2756752H1	3308	3580	96	60209492U1	1489	2005
96	70839657V1	2744	3193	96	70837859V1	3306	3896	96	60209480U1	1489	1845
96	70657566V1	2815	3435	96	495759F1	3326	3893	96	7006928H1	1535	1890
96	2367903F6	2842	3294	96	1332986T6	3341	3399	96	70375676D1	1644	2122
96	2367903H1	2842	3079	96	3297117H1	3428	3687	96	70375660D1	1684	1921
96	2598088T6	2849	3428	96	g2959267	3429	3904	96	494011R1	1689	2115
96	2370164T6	2863	3400	96	g3836196	3462	3898	96	494011R6	1689	2062
96	7339911H1	2891	3434	96	154612T6	3493	3858	96	494011H1	1689	1921
96	494011F1	2890	3445	96	g2877501	3517	3898	96	g3896433	1698	2078
96	70658592V1	2911	3534	96	g3838447	3541	3898	96	994895R1	1733	2255
96	1398685H1	2935	3187	96	g1187596	3595	3893	96	994895H1	1733	2006
96	70656953V1	2938	3555	96	7004590H1	3601	3894	96	680754H1	1778	2047
96	1380634H1	2939	3189	96	6192804H1	3620	3893	96	70374292D1	1783	2008
96	5138789H1	2949	3229	96	6194735H1	3620	3893	96	70375422D1	1783	2241
96	70655570V1	2957	3552	96	6194703H1	3619	3877	96	70375842D1	1789	2222
96	5067594H1	2958	3147	96	495759T6	3642	3849	96	3488220H1	1791	2075
96	494011T6	2971	3405	96	g4737879	3648	3893	96	71220762V1	1851	2480
96	g2051891	2978	3448	96	g758939	3648	3873	96	1332986F6	1863	2182
96	6850945H1	2986	3519	96	g3895731	3648	3898	96	71221685V1	1859	2529
96	g4738083	2987	3447	96	g1079906	3666	3944	96	1332986H1	1863	2097
96	6489656H1	2997	3108	96	767355H1	3801	4056	96	70838337V1	1962	2525
96	g3419253	3004	3445	96	g1115031	3911	4343	96	1964279H1	1965	2251
96	g5656805	3025	3445	96	7337250H1	1	574	96	71221831V1	2034	2530
96	g1444847	3030	3446	96	248552H1	46	257	96	70375269D1	2043	2527
96	g3416160	3047	3449	96	7235980H1	61	574	96	g1357787	2095	2714
96	g1719354	3058	3454	96	g3869258	202	4338	96	5874572H1	2106	2365

96	71221729V1	2117	2580	97	60264804D1	680	1220	99	3016435T6	183	759
96	70838208V1	2146	2490	97	60264819D1	680	1192	99	2094922H1	268	318
96	2598088F6	2168	2790	97	g2011093	754	943	99	4587201H1	585	849
96	2598088H1	2168	2286	97	069791H1	807	940	99	4762091H1	613	811
96	g573567	2168	2536	97	4916612H1	819	1108	99	1495146R6	616	1100
96	6409839H1	2218	2783	97	4916612F7	828	1360	99	1495146H1	616	775
96	5035552H1	2282	2539	97	4719538F6	925	1431	99	5326946H1	671	928
96	70658011V1	2283	2794	97	6337692H1	300	461	99	1495146T6	681	1055
96	70656808V1	2299	2887	97	3371311H1	544	811	99	3703757H1	702	1004
96	1997937R6	2308	2826	97	3091243H1	1164	1445	99	2948181T6	719	1062
96	1997937H1	2308	2587	97	3915431H1	1199	1503	99	2948181F6	726	1100
96	g3887783	2344	2793	97	1954644H1	1145	1429	99	2948181H1	726	983
96	1997937T6	2397	2962	97	2596935H1	1269	1503	99	g4620028	745	1100
96	g1280977	2404	2950	97	4719538T6	1597	1835	99	g928615	748	1102
96	155875H1	2404	2627	97	g812717	1598	1774	99	677653H1	1004	1100
96	155875R6	2404	2906	97	60100951B1	1279	1689	100	5901489T6	1	584
96	70839485V1	2403	2962	97	2410012H1	1308	1528	101	2025051T6	1192	1583
96	6096636H1	2466	2679	97	5781437H1	1353	1643	102	g2932743	1	465
96	7126727H1	2484	3045	97	1499912H1	1387	1582	102	7165328H1	224	722
96	5876310H1	2484	2804	97	2777329H1	1412	1639	102	g5664446	237	597
97	4719538H1	925	1185	97	60100952B1	1415	1827	102	2863343H1	346	643
97	4911350H1	964	1239	97	g2577280	1497	1671	102	4030732H1	346	455
97	1903472H1	984	1228	97	g3648252	1574	1704	102	2863343F6	346	769
97	3639862H1	1027	1306	97	g1128730	1628	1854	102	3323244H1	597	856
97	4541862F6	1034	1422	97	g2837364	1650	1832	103	4987160F6	1	601
97	6907459H1	1061	1564	97	60100950D1	860	1177	103	4986076H1	251	398
97	g5657760	1076	1506	97	60100952D1	860	1116	103	2753343R6	273	678
97	6908516J1	1077	1642	97	1264982H1	895	1131	103	2753343H1	273	523
97	4697720H1	1125	1372	97	6120149H1	908	1402	103	4793432H1	351	482
97	4238385H1	1	283	97	6119056H1	908	1530	103	4793224H1	353	635
97	3403365H1	73	315	97	60264817D1	625	1184	103	2753343T6	394	811
97	3403365F6	74	334	97	60100951D1	637	929	103	4987160H1	1	271
97	6907459J1	151	705	98	6197337H1	1	377	103	3343341T6	430	814
97	4551324H1	169	418	98	6198216T8	1	273	103	4228058H1	434	708
97	3374712H1	211	466	98	6197337F8	18	368	103	864677H1	614	856
97	60264809D1	228	424	98	6331250H1	213	825	103	2755913T6	618	813
97	1954023H1	257	493	99	3016435F6	1	256	103	2755913H1	618	848
97	g792057	641	899	99	3016435H1	1	138	103	2755913R6	618	847
97	60264821D1	680	1235	99	5964071H1	28	318	103	1971855T6	627	812

103	6161690H1	661	856	105	70523321V1	2540	2944	106	3173959T6	1584	2175
104	1006355H1	6597	6859	105	9875595	2611	2954	106	2298374R6	1623	2077
104	3957372H2	6610	6875	105	70523241V1	2671	2938	106	2298374H1	1623	1895
104	186558R6	7598	8102	105	9561027	2772	2938	106	1361272F1	1657	2216
104	94838144	307	6193	105	9875594	1746	2106	106	1361366H1	1657	1834
104	93847721	8369	8524	105	9669502	1745	1996	106	1683557F6	1671	2227
104	93861906	8369	8524	105	70525213V1	1780	2434	106	1683573F6	1671	2086
104	185105H1	7598	7794	105	70522841V1	1864	2410	106	1683573H1	1671	1907
104	186558H1	7598	7745	105	70524066V1	1885	2366	106	1336611H1	1698	1941
104	186558T6	7859	8487	105	70524543V1	1916	2434	106	71265365V1	1447	1892
104	91635936	7988	8197	105	7238311H1	1931	2473	106	70062844V1	1469	1865
104	4619766H1	8062	8337	105	70522425V1	1952	2725	106	2273538H1	1494	1763
104	7356046H1	8121	8524	105	70525244V1	2005	2625	106	3022474H1	1540	1833
104	93797365	8183	8524	105	70526939V1	2013	2610	106	71120676V1	1546	1932
104	6432853H1	8263	8530	105	70526388V1	2192	2409	106	70524882V1	1559	1725
104	6434079H1	8263	8530	105	70524889V1	2198	2767	106	70528634V1	558	1205
104	756968R6	8287	8524	105	70526612V1	2200	2358	106	2408847H1	1882	2132
104	756968H1	8287	8518	105	70522907V1	2239	2973	106	70059242V1	1888	2222
104	9184038	1	8521	105	70522785V1	2248	2927	106	95855887	1901	2223
104	7000401H1	343	428	105	70526373V1	2260	2416	106	95858285	1901	2224
104	1608059F6	445	836	105	7086069H1	2358	2898	106	3409241T6	1911	2367
104	1608059H1	445	664	105	7071063H1	1	547	106	5669576H1	1931	2121
104	2169635F6	1141	1544	105	9913241	159	2181	106	70062956V1	1793	2090
104	2169635H1	1141	1373	105	96299529	164	599	106	70061871V1	1800	2151
104	2169635T6	1716	2134	105	7091369H1	245	847	106	4778510H1	1802	2080
104	7229203H1	2112	2612	105	7347105H1	266	460	106	70061698V1	1811	2222
104	9314259	4291	4813	105	5312260H1	357	578	106	94901915	1816	2222
104	3960183H1	6436	6705	105	7090770H1	1143	1683	106	70060889V1	1815	2268
105	70524885V1	1630	2395	105	70525682V1	1204	1896	106	1623591T6	1819	2177
105	70524843V1	1639	2253	105	70526748V1	1205	1699	106	1915736T6	1818	2176
105	6643169V1	1689	1909	105	2707682H1	1213	1477	106	93155476	1821	2208
105	70646269V1	1689	1909	105	2707682F6	1213	1439	106	95392637	1826	2222
105	70525307V1	1716	2188	105	70522990V1	1214	1837	106	70061837V1	1835	2222
105	9880693	1746	2259	105	1569986H1	1252	1374	106	1623591F6	1853	2222
105	70533384V1	2383	2603	105	70529862V1	1437	1903	106	1623591H1	1853	2074
105	2707682T6	2410	2903	105	70522200V1	1481	2029	106	92198272	1858	2220
105	70523481V1	2420	2965	105	70525480V1	1546	2185	106	960516R1	1859	2222
105	70525826V1	2426	2939	105	6449560H1	1564	2157	106	70062540V1	1860	2222
105	70525835V1	2426	2939	106	3408296H1	1562	1812	106	960516H1	1859	2160

106	960387T1	1859	2185	106	g3151378	1960	2226	106	70061612V1	1322	1882
106	2086141H1	1864	2127	106	g1980460	1995	2313	106	70059581V1	1322	1815
106	4239442T8	1884	2294	106	223027R1	2064	2222	106	70058920V1	1322	1762
106	5376913H1	683	950	106	223027H1	2063	2222	106	70060883V1	1322	1729
106	2557738H1	650	892	106	223027F1	2064	2222	106	2298374T6	1735	2182
106	2560627H1	650	911	106	70529734V1	461	579	106	70061519V1	1747	2333
106	1853191F6	1706	2218	106	g850466	493	824	106	3021149H1	1758	2047
106	1853191H1	1706	1960	106	70530256V1	467	1041	106	g2879046	1775	2225
106	1632340H1	1705	1922	106	3751266F6	1	358	106	18533191T6	1781	2376
106	614199H1	1706	1933	106	3751266H1	1	296	106	g2444573	1794	2221
106	70059326V1	1719	2222	106	3321818F6	103	497	106	3934758F6	895	1411
106	1683573T6	1725	2175	106	3321818H1	104	350	106	70527491V1	846	1499
106	3751266T6	1725	2187	106	6351045H2	329	660	106	71119165V1	884	1492
106	3321818T6	1730	2185	106	71265775V1	801	1368	106	71265279V1	889	1575
106	70062398V1	1341	1862	106	4177726H1	808	1103	106	71264808V1	753	1263
106	71118139V1	1346	1909	106	5296714H1	766	1037	106	71265724V1	731	1013
106	1865168H1	1348	1605	106	7059995H1	799	1271	106	1915736H1	731	969
106	71294927V1	1403	1816	106	096309H1	1101	1323	106	71265549V1	731	1202
106	71120506V1	1404	1857	106	71118507V1	1118	1700	106	71266530V1	732	1246
106	71118652V1	1408	2023	106	71120385V1	1121	1448	106	2681128F6	695	1180
106	71266623V1	1448	1751	106	g2198304	1133	1220	106	g2541182	2106	2424
106	71265047V1	731	1261	106	71119740V1	1134	1424	106	g3039868	2147	2225
106	71119759V1	731	1234	106	4174878H1	1137	1420	106	3931985H1	896	1182
106	71266124V1	732	1366	106	71117317V1	1153	1724	106	3934538H1	895	1200
106	71118641V1	731	1311	106	71119085V1	1197	1825	106	3934758H1	895	1195
106	71118731V1	731	1292	106	3792653H1	1224	1433	106	39343357H1	895	1200
106	71265792V1	731	1260	106	6329373H1	1235	1780	106	3662984H1	1006	1254
106	1915736R6	731	1137	106	71119545V1	1267	1954	106	70529227V1	1009	1601
106	2681128H1	696	984	106	71120838V1	1275	1741	106	71265619V1	1031	1689
106	70531848V1	706	1260	106	4717156H1	1277	1501	106	5946829H1	1044	1325
106	71264971V1	725	1481	106	5391806H1	1282	1481	106	71266345V1	1097	1749
106	71266413V1	922	1608	106	71118747V1	1295	1960	106	71120487V1	1102	1594
106	71117117V1	921	1580	106	70527304V1	1301	1969	106	71266010V1	1097	1765
106	3672409H1	937	1168	106	71120713V1	1306	1885	106	5947885H1	1100	1363
106	71118560V1	968	1537	106	71118117V1	1311	1871	106	3173959H1	1322	1589
106	71119741V1	973	1452	106	5290693H1	1314	1610	106	70059533V1	1322	1585
106	3964084H1	1006	1294	106	5287845H1	1314	1441	106	g1981676	1322	1654
106	3628624H1	1936	2251	106	70530468V1	1320	1851	106	70062034V1	1326	1794
106	g2969311	1938	2222	106	3173959F6	1322	1886	107	5913661H1	1	283

107	5913661F8	1	569	111	g3040122	248	503	115	g990246	1645	1912
107	5913661F6	1	575	111	g3051904	251	501	115	957795H1	1653	1903
107	5913661T6	213	821	111	g3804542	368	750	115	472488H1	1653	1882
108	6796436H1	1	435	111	5282615F6	635	1106	115	472488R1	1654	2125
108	g5837313	111	559	111	5282615H1	884	1106	115	302216H1	1655	1877
109	2536867F6	918	1430	112	g2563121	1	166	115	1876833H1	1654	1913
109	2536867H2	918	1187	112	2792728F6	1	440	115	2697195H1	1656	1898
109	g1926046	932	1379	112	2792728T6	1	417	115	5377444H1	1666	1918
109	g1925836	933	1201	112	g3040744	1	338	115	2431879H1	1668	1914
109	3244923F6	993	1220	112	g2714186	1	401	115	777024H1	1669	1901
109	3244923H1	993	1213	112	7157205H1	1	461	115	3236114H1	1675	1928
109	2878889H1	1010	1304	112	g4371924	71	205	115	1641892H1	1677	1880
109	2878889F6	1010	1349	112	1394888T6	90	304	115	5569350H1	1678	1920
109	g858504	1080	1411	112	1624877H1	94	288	115	3857806H1	1686	1976
109	5567006H1	1081	1257	112	2792728H1	147	439	115	g2751499	1694	2029
109	1896278H1	1106	1341	113	g2943715	1	1450	115	9876527	1697	2020
109	g784668	1209	1286	113	6487571H1	657	1161	115	4069649H1	1708	1834
109	3364252F6	1	432	113	6487571F9	657	1207	115	4307481H1	1724	1900
109	3364252H1	1	226	113	70681361V1	692	1244	115	031299H1	1750	1920
109	096666H1	66	233	113	70681601V1	692	1178	115	3623362H1	1779	2032
109	096675H1	67	240	113	1544823R6	692	1181	115	g5036497	1858	2136
109	7132087H1	255	624	113	70681277V1	692	1164	115	3624213H1	1872	2081
109	4030547F8	461	1001	113	1544823H1	692	898	115	g2411009	1907	2079
109	4030547H1	462	715	113	g4686743	879	1327	115	4721623H1	1918	2011
109	5077091H1	800	1072	113	70680264V1	950	1079	115	2839772H2	1922	2209
110	2717953H1	1	259	113	6476403H1	998	1525	115	6056374H1	1933	2132
110	2806157F6	27	606	114	6272292H2	1	507	115	6056674H1	1933	2136
110	2806157H1	26	323	114	5910821T8	365	636	115	3802178H1	1933	2135
110	2724233T6	367	954	114	5910821T9	365	662	115	4371036H1	1940	2136
111	166942F1	1	624	114	5910821F8	365	793	115	412662H1	1939	2136
111	g3755789	134	505	114	5910821H1	365	676	115	1907478H1	1950	2136
111	g3109437	134	208	115	1551035H1	1625	1845	115	5050475H1	1966	2136
111	g3037830	135	509	115	2716914H1	1625	1873	115	6447704H1	1966	2106
111	g3180013	139	583	115	4876737H1	1631	1915	115	g3888474	1988	2321
111	g4270829	142	429	115	7091270H1	1631	1955	115	5379172H1	1989	2238
111	g3594985	142	562	115	674945H1	1636	1903	115	5015647H1	1994	2136
111	5282615T6	168	751	115	670546H1	1636	1756	115	3327673H1	1995	2251
111	g2942533	248	563	115	2119143H1	1637	1891	115	3567634H1	2021	2137
111	g2953832	248	509	115	2815231H1	1643	1910	115	1739210H1	2025	2215

115	880124H1	2032	2132	115	4696530H1	245	433	115	2886241H1	1493	1744
115	2354054H1	2037	2132	115	928643H1	289	554	115	1222871H1	1495	1739
115	1235532H1	2060	2132	115	4529248H1	370	618	115	4193226H1	1498	1707
115	2350867H1	2062	2132	115	3942806H1	418	694	115	7065251H1	1500	2097
115	9983285	2072	2421	115	7280921H1	466	677	115	2152140H1	1501	1772
115	4190021H1	2084	2132	115	92013891	480	698	115	3763167H1	1502	1559
115	92017399	2116	2329	115	92013455	480	709	115	3571390H1	1504	1790
115	6550613H1	2153	2494	115	5422141H1	481	726	115	942026H1	1509	1756
115	5195955H1	2155	2420	115	3110608H1	525	776	115	4533306T1	1513	2067
115	3907635H1	2185	2464	115	5659913H1	620	896	115	2124615H1	1517	1816
115	93166884	2193	2356	115	5545664F8	647	982	115	3684042H1	1522	1808
115	9994523	2232	2476	115	5545664F6	647	1087	115	6715567H1	1523	2096
115	91099949	2244	2491	115	3163564H1	751	1011	115	3322645H1	1523	1782
115	9757333	2245	2513	115	761584H1	768	982	115	91873672	1524	2006
115	4534796H1	2255	2463	115	6887167J1	837	1454	115	3590863H1	1530	1809
115	1543657H1	2262	2468	115	1852303H1	1011	1084	115	60123902B1	1538	2104
115	4754806H1	2295	2496	115	6948478H1	1087	1504	115	1002539H1	1538	1639
115	1947635H1	2336	2483	115	4158605H1	1206	1469	115	6077425H1	1541	1858
115	3930538H1	2360	2496	115	5270074H1	1238	1440	115	6513731H1	1548	2079
115	2760689H1	2377	2496	115	2365846H1	1239	1469	115	3785763H1	1548	1837
115	5563236H1	2404	2496	115	2444605H1	1244	1464	115	538349H1	1550	1775
115	4310883H1	2408	2496	115	3115706H1	1255	1482	115	1832140H1	1551	1753
115	2778679H1	2439	2496	115	6728257H1	1349	1938	115	1669989H1	1553	1767
115	725638H1	1	243	115	5717548H1	1401	1922	115	450252H1	1553	1773
115	6733284H1	96	640	115	5796003H1	1427	1849	115	737082H1	1556	1778
115	3096672H1	98	410	115	779492H1	1436	1699	115	7065802H1	1560	2096
115	7034622H1	100	606	115	2279382H1	1436	1702	115	6741001H1	1563	2068
115	9777461	120	197	115	5370473H1	1439	1656	115	835332H1	1566	1869
115	5198834H1	146	385	115	4152676H1	1450	1712	115	1599938T6	1569	2099
115	2394482H2	157	372	115	60123909B1	1458	2086	115	4226362H1	1573	1848
115	4970513H1	161	431	115	6960268H1	1466	1902	115	529930H1	1578	1721
115	4969579H1	160	361	115	3449619H1	1461	1706	115	4771124H1	1577	1846
115	377654H1	163	374	115	7039654H1	1461	1970	115	4715169H1	1583	1859
115	305879H1	187	444	115	3890456H1	1461	1758	115	4533768T1	1584	2101
115	307158H1	189	433	115	6966324H1	1466	2051	115	4895425H1	1591	1861
115	3319063H1	215	496	115	765511H1	1469	1812	115	4348930H1	1593	1852
115	5504343H1	219	449	115	4940482T9	1481	2031	115	4348733H1	1594	1854
115	3513751H1	230	473	115	6409578H1	1486	1978	115	92616416	1596	1833
115	4775727H1	230	504	115	6734637H1	1495	1912	115	5698825H1	1596	1847

115	2288814H1	1597	1844	119	3437984H1	274	530	122	60110854B2	1164	1287
115	5762293H1	1598	2136	119	2544176H1	332	520	122	g2884782	1	452
115	3082229T6	1601	1987	120	2807456F6	1	508	122	g2220423	1	398
115	3737781H1	1598	1897	120	2807456H1	1	249	122	g1267721	1	282
115	883098H1	1600	1835	120	2807456T6	122	671	122	g3918260	1	411
115	5336847H1	1601	1837	121	g3595066	1	357	123	2807456F6	194	701
115	2741220H1	1601	1860	121	4665764H1	1	257	123	2807456H1	1	249
115	880546H1	1600	1841	122	70151773V1	587	917	123	2807456T6	31	580
115	6398288H1	1601	1744	122	60203477U1	266	822	123	270567H1	78	161
115	3252954H1	1608	1863	122	522228H1	955	1199	123	269931H1	374	499
115	3779658H1	1618	1921	122	522135H1	955	1090	123	269626H1	1824	2053
116	g1891130	669	957	122	026879H1	768	1022	123	269080H1	128	352
116	7333578H1	1	523	122	60203621U1	1	548	123	270403H1	85	349
116	6545439H1	141	676	122	60203622U1	118	507	123	270910R1	1824	2022
116	g3805536	534	966	122	4401727H1	579	846	124	587588R6	220	555
116	g3322110	534	772	122	g2540618	526	801	124	587588T6	44	555
116	5610773H1	537	788	122	2807905H1	484	793	124	587588H1	1	165
117	6929774H1	1	513	122	70152547V1	797	1219	125	3321035F6	317	786
117	6052078J1	72	520	122	3812508H1	140	438	125	3321035H1	330	595
117	6052078H1	72	520	122	g1265991	198	338	125	g1319620	414	927
117	4970577H1	120	381	122	3860472H1	256	546	125	g2741801	414	556
117	4970577F6	120	483	122	3520754H1	334	621	125	g2841030	1354	1422
117	6292129H1	423	637	122	70152228V1	391	1018	125	g2933104	421	906
117	6294687H1	423	647	122	4350225H1	364	638	126	5259815H1	1	206
117	2807905H1	555	863	122	70155823V1	419	986	126	3568526H1	71	366
117	g2540618	597	871	122	g3919706	1	424	126	1289824F6	181	734
117	4401727H1	650	916	122	2512390F6	1	316	126	g928730	693	889
117	5729803H1	731	1236	122	g1792877	1	365	126	764159H1	693	849
117	g1301257	787	1245	122	g4187765	4	446	126	6620992H1	718	1297
117	026879H1	838	1092	122	g2905531	4	102	126	839936R1	825	1369
117	g1303063	897	1111	122	70156040V1	960	1356	126	1289824H1	181	349
117	522135H1	1025	1160	122	70151954V1	828	1353	126	839936H1	825	1066
117	522228H1	1025	1269	122	2512390H1	14	316	126	3869224H1	996	1286
118	587588R6	1	336	122	60202389B1	858	1335	126	1685280F6	522	955
118	587588T6	1	512	122	60202388B1	870	1329	126	3223525H1	1137	1457
118	g1069975	229	539	122	60202388B2	924	1329	126	3843717H1	1	293
119	g2809760	1	443	122	999391H1	37	270	126	g1395923	1198	1531
119	g2934256	68	387	122	2512390T6	38	315	126	1685280H1	522	754
119	2785236H2	220	481	122	4970577T6	45	618	126	5028090H1	1333	1598

126	4216695H1	1404	1656	130	4032140H1	1443	1700	131	6881955J1	2681	3266
126	1289824T6	249	852	130	1597096H1	1442	1628	131	2825680F6	7992	8265
126	92540596	600	897	130	3438058T6	1483	1957	131	95036098	8059	8265
126	5724304H1	664	1233	130	4032240T9	1491	1900	131	4027974T6	6903	7418
126	1947742T6	688	858	130	93804916	1564	1997	131	5717756H1	6914	7382
126	91225270	688	889	130	93432508	1574	2002	131	2717848T6	6942	7421
126	3438058F6	269	562	130	93254781	1621	2002	131	4722802H1	6965	7069
126	3438058H1	318	562	130	1947742T6	689	861	131	93594812	7015	7468
126	3438058T6	45	516	130	9928730	693	892	131	6403421H1	7053	7317
126	93804916	6	436	130	3223525H1	1152	1474	131	93163456	7067	7469
126	93254781	1	379	130	5259815H1	1	206	131	2649733F6	7123	7465
127	3504571H1	910	1216	130	3568526H1	71	366	131	2649733H1	7123	7369
127	92055741	1038	1358	130	1289824F6	181	737	131	7003170H1	7124	7465
127	91270278	1025	1374	130	1289824H1	181	349	131	9683322	7127	7465
127	2733544H1	1137	1405	130	92540596	600	900	131	2649733T6	7128	7424
127	91162686	1144	1490	130	4216695H1	1421	1676	131	2484677H1	7142	7369
127	91109059	1197	1484	130	3438058F6	1437	1733	131	96075627	7147	7469
127	91774715	1212	1519	130	3438058H1	1437	1682	131	93048962	7149	7468
127	93897241	1316	1718	130	1597096F6	1442	2036	131	212391H1	7200	7437
127	5854467H1	1401	1557	130	1685280H1	1243	1477	131	96506945	7080	7465
127	91898243	1492	1691	130	5028090H1	1348	1616	131	96073337	7082	7469
128	92358498	616	997	130	7213258H1	1225	1803	131	92630574	7111	7470
128	183176H1	747	971	130	91395923	1213	1549	131	5207126H2	2813	2981
128	183176R6	507	971	130	3928775H1	696	891	131	2825680H1	7994	8265
128	183176R1	359	971	130	1289824T6	249	855	131	9560960	8006	8268
128	2733388H1	659	888	130	3843717H1	1153	1448	131	9796025	8011	8277
128	5616358H1	602	878	130	764159H1	693	852	131	1415181H1	8034	8265
128	92824012	433	792	130	5724304H1	664	1213	131	3336518H1	3434	3669
128	94762579	167	600	130	6040888H1	665	891	131	4723427H1	3458	3606
128	7104793H1	1	520	130	91225270	689	892	131	70390598D1	3509	4085
128	4004284H1	207	474	130	7153412H1	656	1189	131	264846H1	2524	2856
129	3928775H1	1	192	130	3640283T9	1701	1932	131	6357277H1	2531	2839
129	2562126T6	40	182	130	3640283T8	1701	1908	131	5544294H1	2708	2828
130	94902006	709	895	130	93538751	1690	2005	131	2101112T6	7755	8220
130	2562126T6	708	852	130	3640283F8	1701	1949	131	7162079H1	218	671
130	839936R1	828	1385	130	6620992H1	717	1277	131	92599501	1	4447
130	839936H1	828	1073	131	3404480H1	1921	2103	131	6044517J1	7709	8237
130	1685280F6	1040	1477	131	5665320H1	2177	2358	131	810518H1	8140	8262
130	3869224H1	1001	1301	131	872922H1	7624	7881	131	2101112H1	7763	8008

131	2101112R6	7764	8137	131	g5675561	3974	4452	131	5036970H1	7696	7961
131	6559424H1	7774	8284	131	6618788H1	3991	4472	131	g2212423	7200	7465
131	g2140984	7774	8165	131	g3144228	4048	4452	131	3282024T6	7255	7409
131	3781662H1	7815	8126	131	g2751511	4080	4441	131	3323350H1	7271	7524
131	744157H1	7818	8046	131	1863153F6	4273	4666	131	g2768029	7301	7465
131	4774224T9	7821	8181	131	1863161F6	4273	4526	131	3322677H1	7313	7591
131	g2324704	7831	8266	131	1863153H1	4273	4520	131	g1515911	7317	7464
131	g3796940	7834	8265	131	385245H1	4275	4498	131	3865622H1	7330	7526
131	g5839127	7843	8265	131	g4970402	4364	4819	131	g317850	7332	7592
131	g3932489	7870	8265	131	5857654H1	4368	4636	131	g3174898	7357	7469
131	g683058	7964	8268	131	g1624652	4395	4466	131	4253321H1	7391	7470
131	g1225252	7984	8268	131	g1023422	4479	4773	131	4244366H1	7390	7465
131	2825680T6	7985	8226	131	g1023318	4511	4801	131	3334656H1	7394	7606
131	3278368H1	7719	7959	131	3341807F6	4527	4888	131	6355568H1	3040	3236
131	4726587H1	7619	7844	131	3341807H1	4527	4766	131	g1881143	3040	3347
131	2108075H1	7405	7654	131	4027974F6	4592	4881	131	1372008H1	7499	7667
131	g1241115	7445	7723	131	4027926H1	4592	4822	131	2292865H1	7499	7721
131	4632120H1	7488	7765	131	6024929H1	4601	4923	131	g915845	7561	7784
131	4632247H1	7488	7765	131	2203255H1	4755	5006	131	2289132H1	7571	7802
131	7154510H1	7499	7643	131	4184071H1	4839	5084	131	5832896H1	7593	7874
131	6162751H1	7497	8049	131	3322055F6	4994	5518	131	4774272H1	7612	7881
131	1593082F6	3583	4007	131	3322055H1	4994	5271	131	1393295H1	7499	7692
131	1593043H1	3583	3803	131	1863161T6	5057	5459	131	1772773H1	7522	7784
131	1593082H1	3583	3803	131	811828H1	5105	5352	131	6269862H1	6136	6658
131	7236349H1	3664	4161	131	789137H1	5167	5226	131	g1975380	6238	6568
131	4004354H1	3665	3798	131	g3077295	5179	5635	131	3389337H1	6336	6619
131	439020H1	3680	3902	131	3242918H1	5346	5610	131	6706684H1	6370	6913
131	3480603H1	3698	3865	131	4578433H1	5545	5787	131	3282024F6	6416	6946
131	g1678362	3734	3890	131	5694130H1	5702	5886	131	3282024H1	6416	6662
131	g4691014	3746	4204	131	4255728H1	5783	6052	131	3854583H1	6428	6705
131	g6132554	3779	4207	131	5673677H1	5815	5974	131	2509302H1	6442	6670
131	g3756265	3780	4206	131	g1880292	5855	6068	131	7225908H1	6547	7110
131	g1199039	3835	4151	131	5768386H1	5903	6448	131	3147875H1	6581	6852
131	g819518	3859	4212	131	g1442274	5974	6178	131	3797563H1	6583	6873
131	2155889F6	3867	4265	131	g1678263	6029	6205	131	g2398342	1058	1351
131	2155889H1	3867	4106	131	3607889H1	6044	6335	131	6881955H1	1921	2185
131	3513325H1	3880	4124	131	g709099	6063	6392	131	2717848F6	6584	7049
131	70391721D1	3896	4321	131	g769480	6063	6274	131	2717848H1	6584	6832
131	2155889T6	3964	4405	131	g692094	6065	6419	131	4058694H1	6629	6895

131	025240H1	6665	6932	133	g5109774	443	863	137	3960046F8	333	926
131	4270965H1	6782	6955	133	g5526440	420	841	137	g4114677	609	1055
131	3341807T6	6814	7417	133	g4599202	303	725	137	g3674688	744	1053
131	3322055T6	6820	7418	133	6306770H1	1	450	137	2705604T6	744	1011
131	4027974T9	6822	7361	134	4186114H1	683	1024	137	g3429159	744	1052
131	g691854	6874	7235	134	4186114F6	683	1091	137	1871586H1	744	869
131	4029613H1	6873	7102	134	4186114T6	921	1587	137	1998855H1	744	926
131	g708808	6874	7171	134	4165023H1	674	955	137	2135293H1	763	1036
131	g565754	6874	7098	135	2268189H1	1	231	137	2135293F6	763	1227
131	124772H1	6888	7030	135	2268189R6	1	379	137	2100630H1	865	1055
132	1630826T6	1136	1410	135	1470335H1	37	230	137	292419T6	874	1001
132	g5813217	1172	1255	135	g2505442	142	353	137	3960046T8	887	1009
132	1631555T6	1194	1410	135	g2505398	157	521	138	6100950H1	1	213
132	3878121H1	1210	1271	135	g2458763	157	579	138	6302544H1	151	468
132	1986029H1	1210	1281	135	1445774H1	262	507	138	6144369H1	166	755
132	2956806H1	1210	1284	135	2268189T6	334	885	138	6144369F8	166	749
132	1372584H1	1214	1417	135	368103H1	520	763	138	6099148H1	208	488
132	854216H1	1	247	135	g2328398	608	929	138	6281896H1	383	528
132	854312R6	1	445	135	2322571H1	690	923	138	6111359H1	570	864
132	854312H1	1	151	136	6114201H1	1	292	138	6028709H1	650	932
132	2465049H1	150	358	136	3287273H1	260	505	138	6144369T8	846	1259
132	2465049F6	150	697	136	g779441	375	522	139	5056523H1	2466	2604
132	2271395H1	192	455	136	g879517	78	440	139	2271032H1	893	1152
132	2271395R6	192	704	136	g870148	135	450	139	4243388H1	919	1251
132	g2184123	196	582	136	g883117	77	465	139	4552488H1	958	1120
132	g1692736	348	740	136	2120815H1	450	565	139	g1953334	885	1086
132	2465049T6	519	1047	136	1631555H1	450	565	139	g1953371	885	986
132	1601994H1	535	677	136	1631555F6	450	565	139	g713204	1029	1191
132	5274102T6	569	1060	136	g705961	450	565	139	g1967839	1091	1429
132	g3413112	652	1088	136	1547031H1	452	565	139	3737077H1	1295	1453
132	g1692707	687	1094	136	4796579H1	455	563	139	2271032R6	893	1370
132	g3428202	689	1088	136	5081193H1	457	508	139	6326476H1	1313	1610
132	g1686359	836	967	136	1630826F6	450	565	139	1845337T6	2212	2566
132	g2714128	860	1089	136	1630826H1	398	546	139	1845337R6	2212	2562
132	g3843958	950	1089	136	g2023471	491	696	139	1845337H1	2212	2433
132	g4394372	950	1088	137	6864812H1	1	502	139	g4288591	2215	2599
132	g3844127	950	1089	137	292419H1	194	469	139	g5659132	2219	2551
132	g661412	957	1252	137	292419R6	196	486	139	g13114H1	2228	2509
133	g5438299	717	1143	137	3960046H2	333	473	139	g1960980	2253	2604

139	95887689	2244	2601	139	2061567H1	1698	1970	140	2271032R6	402	880
139	911966H1	2249	2503	139	6158833H1	1433	1523	140	2271032H1	402	661
139	95838009	2250	2611	139	794690H1	1496	1698	140	4243388H1	428	761
139	95913083	2251	2604	139	6576416H1	1513	1850	140	4552488H1	467	629
139	93741346	2254	2611	139	1889107H1	1542	1812	140	9713204	538	701
139	93785022	2297	2602	139	2250985H1	1734	1964	140	91967839	600	939
139	92463906	2312	2607	139	1889107F6	1542	1892	140	3737077H1	805	963
139	94312734	2347	2599	139	4399135H1	1734	1991	140	6326476H1	823	1120
139	92100983	2358	2591	139	4399331H1	1734	2000	140	91968949	855	1322
139	4519262H1	2360	2599	139	1501428H1	1737	1930	140	92229641	870	1302
139	1272843T6	2368	2562	139	6823952J1	1549	2162	140	476160H1	899	1165
139	6104909H1	2413	2604	139	6018675H1	1812	2378	140	6158833H1	943	1033
139	4180584H1	1872	2122	139	4516041H1	1817	2066	140	794690H1	1006	1208
139	91968950	1886	2368	139	1272843H1	1	245	140	1889107F6	1052	1402
139	5038396H1	1888	2146	139	1272843F6	1	593	140	1889107H1	1052	1322
139	6576957H1	1907	2455	139	1272843F1	1	331	140	5840503H2	1088	1345
139	3254138H1	1908	2115	139	1274319H1	21	218	140	2061567H1	1208	1480
139	5946104H1	1910	2145	139	2598183H1	24	263	140	2061567R6	1208	1687
139	2700155F6	1922	2433	139	3256051H1	25	268	140	4399331H1	1244	1510
139	2700155H1	1922	2121	139	6826541J1	33	663	140	4399135H1	1244	1501
139	2061567T6	1951	2554	139	6823903H1	34	188	140	2250985H1	1244	1474
139	3365965H1	1983	2108	139	6820526H1	422	747	140	1501428H1	1247	1440
139	95591869	2006	2220	139	6820526J1	422	747	140	4516041H1	1327	1576
139	1749882T6	2039	2571	139	6825541H1	672	1240	140	4180584H1	1382	1632
139	1752020H1	2049	2258	139	3857549H1	797	1106	140	91968950	1396	1878
139	2700155T6	2063	2563	139	1749882F6	811	1171	140	5038396H1	1398	1656
139	1889107T6	2099	2562	139	1749882H1	811	1085	140	3254138H1	1418	1625
139	3236592H2	2125	2340	139	6829715H1	860	1357	140	5946104H1	1420	1655
139	5291302H1	2152	2398	140	1272843F1	1	331	140	2700155F6	1432	1943
139	95038163	2168	2608	140	1272843F6	1	587	140	2700155H1	1432	1631
139	92355286	2200	2589	140	1272843H1	1	245	140	2061567T6	1461	2064
139	93146568	2200	2604	140	1274319H1	21	218	140	3365965H1	1493	1618
139	5307668H1	1567	1691	140	2598183H1	24	263	140	1749882T6	1549	2081
139	91968949	1345	1812	140	3256051H1	25	268	140	70366789D1	1569	1883
139	5840503H2	1578	1835	140	3857549H1	306	615	140	1752020H1	1559	1768
139	92229641	1360	1792	140	1749882F6	320	680	140	2700155T6	1573	2073
139	6725031H1	1675	2299	140	1749882H1	320	594	140	1889107T6	1609	2072
139	476160H1	1389	1655	140	91953334	394	595	140	3236592H2	1635	1850
139	2061567R6	1698	2177	140	919533371	394	495	140	5291302H1	1662	1908

140	g2355286	1710	2099	143	6338721H1	60	530	147	7008530H1	19	463
140	g3146568	1710	2114	143	3722062H1	63	355	147	7161666H1	34	435
140	1845337R6	1722	2072	143	3564688H1	78	370	147	7017571H1	42	211
140	1845337T6	1722	2076	143	g1390898	122	415	147	3277305H1	128	393
140	1845337H1	1722	1943	143	5154455F6	261	738	147	3565182H1	492	782
140	g4288591	1725	2109	143	5154455H1	489	738	147	g4293857	672	946
140	913114H1	1738	2019	143	5155056H1	492	738	147	2992317H1	816	1097
140	g1960980	1763	2114	143	5440206H1	376	645	147	5643301R6	823	1114
140	911966H1	1759	2013	143	6206972H1	638	1158	147	5643301R8	939	1115
140	g3741346	1764	2121	143	2240790T6	39	495	148	g3742856	2099	2440
140	g3785022	1807	2112	143	g2155919	45	468	148	g1903217	1	2439
140	g2463906	1822	2117	143	3219860T6	39	451	148	7183853H1	1	319
140	g4312734	1857	2109	143	3219860H1	135	451	148	g3665358	2048	2440
140	g2100983	1868	2101	143	3219860R6	1	451	148	6282319H1	2330	2439
140	4519262H1	1870	2109	143	3221716H1	149	451	148	g3931095	2387	2439
140	1272843T6	1878	2072	143	2412827H1	233	450	148	g1487041	2284	2448
140	6104909H1	1923	2114	143	g3932128	5	420	148	g1365146	1897	2439
140	5056523H1	1976	2114	143	g2155881	1	335	148	g1487088	1978	2188
141	2211487F6	1	511	143	g2775288	1	329	148	7183071H1	2	532
141	2211487T6	195	778	143	g3277838	1	325	148	7184162H1	6	440
141	2412148H1	263	497	143	g1390787	5	313	148	7182312H1	265	747
141	2564905H1	540	809	143	2532555T6	50	283	148	g1365200	1875	2451
141	125647H1	559	768	143	g1422648	1	217	149	6807937J1	1	617
141	2242295T6	625	773	143	g1783871	2	194	149	934453R6	169	650
141	2211487H1	1	241	144	60210582U1	1	374	149	934453T6	169	578
142	3404890H1	1	218	144	g857093	1	226	149	934453H1	169	430
142	3387263H1	44	343	144	60124509B1	198	336	150	g2356671	1	436
142	g1225131	45	275	145	g2324899	1	329	150	3878737H1	255	511
142	2745513H1	52	296	145	g4525985	12	329	151	3613937H1	1	286
143	3330364H1	1	264	145	3516332H1	36	278	151	3613937F6	1	551
143	2532555H1	1	245	145	3012696F6	162	329	151	3356375H1	14	272
143	2532555F6	1	202	145	3012696H1	162	337	151	4365653H1	26	279
143	3452732H1	7	223	145	3012696T6	169	288	151	3133682F6	168	468
143	6206478H1	8	551	145	561748H1	231	329	151	3133682H1	168	446
143	2240790H1	7	246	146	6623203J1	1	573	151	2784431H2	322	410
143	2240790F6	7	455	146	3538176T6	590	927	151	4760428H1	378	661
143	4052530H1	9	302	147	5643301H1	1	266	151	g1987179	426	713
143	2211943H1	38	294	147	5643301F6	1	412	151	3985930H1	427	714
143	2207141H1	1	161	147	7007819H1	12	557	151	g3840682	469	880

151	6619463H1	506	1081	154	5950630H1	125	405	155	3256088H1	24	259
151	93594561	524	876	154	5950410H1	125	401	155	1728945H1	26	246
151	3613937T6	562	835	154	4933727H1	239	384	155	6141236F8	28	633
151	6148958H1	732	1299	154	3533254H1	273	564	155	6141236H1	28	370
151	5067091H1	1	243	154	4875149T6	344	737	155	6328822H1	47	592
151	2583002H1	33	298	154	93202357	457	784	155	3388023H1	89	361
151	2583002F6	33	533	154	748032H1	574	776	155	6134014H1	110	401
151	9273554	104	290	155	3751233H1	577	867	155	3323733H1	192	461
152	70255820V1	1	485	155	5812189H1	644	840	155	3409854H1	357	618
152	70249242V1	70	324	155	5812190H1	644	845	155	4068325F6	515	1108
152	70254597V1	114	627	155	311752H1	657	744	155	4068325H1	517	804
152	70254754V1	114	607	155	373638H1	663	880	155	3629455H1	560	835
152	70254688V1	137	517	155	6333709H1	705	1240	156	7006557H1	1	488
152	70255790V1	244	565	155	6329688H1	705	1325	157	6459173H2	4960	5300
152	70255539V1	507	565	155	7080559H1	760	1170	157	71034189V1	3224	3885
152	70255201V1	514	565	155	6532643H1	803	1384	157	71033929V1	3308	3912
153	94194301	1	458	155	6141236T8	989	1579	157	92166710	3325	3492
153	93244448	3	471	155	4068325T6	1018	1641	157	71239210V1	3365	4028
153	93804087	5	421	155	7068876H1	1069	1483	157	71035047V1	3406	3932
153	2383032T6	46	518	155	3256362H1	1308	1554	157	71035847V1	3407	3932
153	70046632V1	217	736	155	91400213	1337	1682	157	71239003V1	3407	3934
153	70046986V1	102	652	155	93254782	1346	1682	157	7252515J2	3410	3964
153	70047134V1	98	553	155	91383466	1395	1696	157	617587H1	3462	3716
153	6208108H1	1	540	155	6412487H1	1494	1931	157	617587R6	3464	3954
153	70046575V1	96	533	155	5534143H1	1578	1820	157	71035452V1	3487	4142
153	70047390V1	97	515	155	6869946H1	1717	2275	157	71239589V1	3548	4177
153	2383032F6	1	492	155	94510725	1831	2272	157	71034449V1	3565	4148
153	4938245H1	119	406	155	6298042H1	1871	2146	157	71036467V1	3574	4249
153	70047509V1	11	414	155	91998847	1973	2275	157	71019079V1	3590	4079
153	2383032H1	1	228	155	3541104H1	1991	2272	157	71034628V1	3600	4175
153	1952540H1	1	157	155	5499626H1	1	255	157	71033566V1	3603	3776
153	70046695V1	11	94	155	5500309H1	1	227	157	71033563V1	3605	4306
154	750543H1	1	183	155	5500026H1	1	161	157	71033740V1	3642	4142
154	91474315	42	323	155	5499909H1	1	206	157	71034052V1	3692	4322
154	3722596H1	61	333	155	2723940H1	1	243	157	2852347H1	3699	3792
154	4875149F6	70	195	155	2723940F6	1	333	157	71034523V1	3759	4400
154	4875149H1	70	257	155	5643224H1	22	276	157	71021626V1	3780	4091
154	6561179H1	71	633	155	3126704H1	22	298	157	71033276V1	3784	4394
154	2029094H1	121	372	155	91998848	22	296	157	71035439V1	3832	4224

157	71239794V1	3841	4296	157	71036110V1	2922	3457	158	70479313V1	3061	3562
157	71036851V1	3933	4532	157	71036841V1	2925	3050	158	70480477V1	3075	3701
157	2902436H1	4020	4325	157	71238907V1	3000	3542	158	70480157V1	3098	3662
157	71035620V1	4052	4596	157	71033235V1	3023	3512	158	4564168H1	3087	3365
157	617587T6	4094	4694	157	71239855V1	3037	3682	158	70466004V1	3018	3732
157	71240672V1	4243	4499	157	71035104V1	3060	3655	158	70478380V1	3019	3638
157	92839119	4251	4718	157	71240132V1	3064	3600	158	4557976H1	3015	3240
157	71239718V1	4264	4729	157	71022089V1	3064	3174	158	3618747H1	1905	2177
157	71240638V1	4263	4415	157	71020694V1	3064	3173	158	6510691H1	1907	2199
157	92716554	4274	4723	157	71033750V1	3068	3576	158	6510634H1	1907	2192
157	2839872F6	4473	4873	157	71036780V1	3070	3575	158	6510739H1	1907	2183
157	2839872H2	4473	4724	157	71240343V1	3074	3590	158	4554621H1	2044	2312
157	6701187H1	4517	5071	157	71035904V1	3096	3656	158	70464785V1	2168	2732
157	71033989V1	4552	4713	157	71239106V1	3098	3535	158	4554554F6	2168	2649
157	6700916H1	4758	5106	157	71239593V1	3107	3663	158	4554554H1	2168	2409
157	91353781	1	3523	157	71036268V1	3116	3650	158	70466681V1	2298	2736
157	7183461H1	8	510	157	71033766V1	3130	3794	158	70467583V1	2823	3476
157	5878868F8	38	589	157	71036727V1	3136	3763	158	70480720V1	2857	3511
157	5878868T9	38	467	157	71036255V1	3150	3690	158	70479363V1	2850	3206
157	5878868H1	40	300	157	3246035H1	3163	3414	158	70476260V1	2859	3447
157	6621194J1	465	1063	157	71236782V1	3177	3581	158	70481657V1	2790	3146
157	7044151H1	1537	2130	157	71034117V1	3225	3899	158	70466450V1	2775	3513
157	7115836H2	1721	2207	158	2702437H1	3382	3692	158	70476879V1	2765	3450
157	6891967H1	1744	2304	158	2028546H1	3382	3674	158	70477920V1	2806	3426
157	7112010H2	2069	2273	158	70469621V1	3384	3974	158	70466934V1	2704	3432
157	7115993H2	2217	2833	158	70477624V1	3362	3551	158	70465560V1	3293	3609
157	71036402V1	2382	2971	158	70464393V1	3358	3664	158	70476467V1	3297	3594
157	71036528V1	2382	2844	158	2028546R6	3382	3697	158	70479516V1	3309	3697
157	71036302V1	2382	2836	158	897912H1	3532	3695	158	4792962H1	3311	3605
157	5682881F6	2382	2755	158	2657383H1	3542	3682	158	70467134V1	3311	3697
157	5682881H1	2382	2556	158	70466432V1	3600	4154	158	4554470H1	3317	3600
157	7121747H1	2421	3009	158	3405032H1	2336	2592	158	70466403V1	3271	3697
157	6554467H1	2459	2877	158	3099417H1	2407	2687	158	70480199V1	3270	3697
157	71035730V1	2859	3427	158	70467835V1	2535	3117	158	70469816V1	3293	3654
157	71034930V1	2861	3427	158	3936618H1	2535	2799	158	70468353V1	3282	3697
157	5693974H1	2871	3141	158	70468637V1	2538	3232	158	70481137V1	3205	3697
157	71033786V1	2880	3524	158	70465732V1	3029	3708	158	70466975V1	3210	3697
157	71239770V1	2904	3495	158	94568142	3029	3550	158	70466049V1	3236	3697
157	71036410V1	2922	3457	158	70477055V1	3050	3634	158	70478359V1	3235	3666

158	6515587H1	3242	3671	158	70478180V1	2901	3570	159	3028782F6	505	841
158	70478211V1	3217	3682	158	70465864V1	2900	3534	159	3028782H1	505	807
158	4558722H1	1289	1534	158	70479279V1	2923	3570	159	1005592H1	599	889
158	3618747F6	1904	2401	158	70476782V1	2927	3466	159	4646393H1	801	1061
158	9190751	50	3497	158	70469166V1	2935	3628	159	5278286H1	912	1125
158	91736195	979	1234	158	70476816V1	2992	3529	159	2521969F6	950	1354
158	2624295R6	1016	1412	158	70480392V1	2746	3208	159	983403H1	1063	1335
158	2624295H1	1016	1225	158	70478225V1	2751	3368	159	983403R6	1064	1315
158	70466944V1	3104	3692	158	70478182V1	3467	4041	159	188949H1	1106	1306
158	70480113V1	3129	3556	158	70498607V1	3496	3697	159	983403T6	1123	1347
158	70468718V1	3142	3695	158	70465130V1	3440	3697	159	988925H1	1126	1349
158	70477732V1	3142	3772	158	2301520H1	3457	3705	159	2521969H1	1129	1354
158	70469007V1	3149	3697	158	70467663V1	3171	3800	159	2639257H1	1177	1339
158	70469207V1	3322	3657	158	70479112V1	3208	3697	159	983273H1	1241	1544
158	70480324V1	3326	3682	158	70467111V1	2890	3430	159	3692640T6	1247	1832
158	70466503V1	3328	4068	158	4562810H1	2883	3182	159	4013213H1	1308	1598
158	70479148V1	3328	3697	158	70473226V1	2882	3252	159	3877453H1	1331	1614
158	70464268V1	3329	3697	158	2620793H1	2886	3178	159	4010336H1	1328	1611
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158	70467082V1	3389	3682	158	70481798V1	2721	3378	159	3011865F6	1340	1761
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158	70469171V1	2699	3374	158	70479545V1	3359	3667	159	3011865H1	1376	1459
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158	70481022V1	2759	3486	159	3685395H1	3	271	159	3958442H1	1377	1474
158	2624295T6	2762	3351	159	3692640H1	4	270	159	5276870H1	1376	1535
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158	70467878V1	2660	3107	159	306745H1	13	386	159	6338013H1	1390	1881
158	70481284V1	2587	3175	159	945423H1	11	293	159	6335720H1	1390	1883
158	70467582V1	2607	3072	159	988248R1	179	383	159	3688195H1	1398	1695
158	70465805V1	2630	3318	159	988248H1	179	291	159	4151882H1	1401	1641
158	70481125V1	2624	3464	159	4454887H1	250	519	159	3874704H1	1439	1714
158	70469427V1	2642	3286	159	3877090H1	350	619	159	5169119H1	1476	1610
158	6287762H2	1	475	159	6901148H1	368	825	159	5278359H1	1475	1705

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159	5277625H1	1723	1973	160	g4080952	4558	4936	160	1901241H1	4719	4976
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159	977605T1	1764	2179	160	g1271129	4575	4962	160	6715734H1	4726	4923
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160	5371320H1	3264	3396	161	5405570H1	2061	2269	161	664952H1	2142	2367
160	4441134H1	3378	3656	161	5451646H1	2063	2243	161	1992383H1	2143	2344
160	9682946	3380	3626	161	92000931	2063	2316	161	3468549H1	2219	2487

161	4256959H1	2161	2426	161	5968971H1	685	1143	161	5857943H1	1354	1638
161	6993692H1	2148	2573	161	1476202H1	762	924	161	7070664H1	1508	1855
161	534218H1	2156	2414	161	865333H1	780	910	161	6416952H1	1519	1822
161	664384H1	2156	2421	161	7193726H2	786	1230	161	6894069H1	1526	2069
161	4713170H1	2177	2420	161	6891594J1	877	1507	161	5013816H1	1533	1783
161	663504H1	2219	2347	161	4031012H1	981	1202	161	533363H1	1538	1775
161	2247226H1	2184	2445	161	6446990H1	988	1498	161	4761822H1	1540	1812
161	2245218H1	2184	2452	161	g6133077	1024	1478	161	2904083H1	1554	1850
161	531779H1	2184	2426	161	3941692H1	1056	1337	161	4306790H1	1577	1687
161	2042975H1	2184	2450	161	4772703H1	1105	1361	161	1991775H1	1578	1797
161	6741472H1	2204	2750	161	g668477	1117	1416	161	4337965H1	1580	1827
161	4490622H1	2204	2747	161	g870488	1118	1438	161	5833237H1	1585	1750
161	4063891H1	2241	2390	161	g698735	1117	1456	161	3469095H1	1597	1855
161	5960583H1	2242	2800	161	g669863	1117	1378	161	4316943H1	1635	1803
161	3781135H1	2190	2495	161	g768138	1143	1468	161	5876761H1	1647	1931
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161	6891449J1	2214	2315	161	g875401	1174	1475	161	2963466H1	1724	2028
161	554022H1	2202	2438	161	1617044H1	1178	1392	161	4316745H1	1724	2020
161	4532533H1	2273	2435	161	5781411H1	1182	1459	161	4315891H1	1744	2022
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161	4691246H1	2208	2424	161	3945042H1	1194	1480	161	4753020H1	1751	2007
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161	5967249H1	2224	2744	161	3466710H1	1203	1464	161	4316639H1	1772	2046
161	6765073J1	2275	2692	161	5860513H1	1221	1490	161	2705655H1	1800	2083
161	666644H1	2212	2384	161	6770719H1	1238	1355	161	5961924H1	1790	2298
161	5579450H1	2212	2365	161	5656957H1	1238	1487	161	4695643H1	1820	2036
161	1618034H1	2212	2418	161	5204013H1	1238	1505	161	2707689H1	1896	2192
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161	6784888H1	439	960	161	5013751H1	1264	1491	161	4342620H1	1992	2246
161	6882093H1	479	990	161	4019484H1	1278	1543	161	6882093J1	1996	2568
161	6882161H1	479	992	161	6063870H1	1281	1564	161	6886296J1	2001	2481
161	6766193H1	480	961	161	3471004H1	1298	1570	161	7253724H1	2024	2531
161	6977268H1	504	925	161	5206590H1	1304	1546	161	2423349H1	2027	2272
161	7230035H1	585	1180	161	6746950H1	1304	1831	161	4316734H1	2032	2342
161	7075220H1	629	1180	161	5857309H1	1303	1576	161	4256579H1	2036	2265
161	1485846F6	646	910	161	5968610H1	1308	1851	161	6763709H1	2023	2568
161	g1975476	664	884	161	g2012398	1308	1663	161	6771870J1	2031	2578
161	1486578H1	676	912	161	3469688H1	1317	1580	161	4772777H1	2046	2149

161	6740527H1	2041	2522	164	5911540F8	1	460	167	5587272H1	18	282
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161	5205527H1	2058	2293	164	5911540T8	78	570	167	1551008H1	18	211
161	5575884H1	2060	2251	165	5905252F8	1	497	167	5347226H1	19	275
161	3466389H1	2061	2306	165	5905252F6	35	576	167	6819761H1	19	616
161	5955966H1	2315	2492	165	5905252H1	35	313	167	5117110H1	20	289
161	5205250H2	2316	2479	165	5905252T6	376	821	167	3447366H2	19	275
161	4776444H1	2311	2566	166	4020439F8	1	391	167	2445594H1	19	249
161	6517485H1	2347	2825	166	2773907F6	1	173	167	4622780H1	19	288
161	2292420H1	2408	2647	166	2773907H1	1	146	167	2111981H1	20	282
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161	6552988H1	2494	2887	166	4020439T8	6	503	167	2461616H1	19	197
161	6552963H1	2494	2887	166	2773907T6	129	435	167	5379341H1	19	275
161	4619412H1	2571	2791	166	g820143	313	435	167	3820792H1	20	310
161	4775811H1	2566	2801	167	6919815H1	1	89	167	4347282H1	21	264
161	g1194667	2595	2887	167	6100456H1	12	272	167	1322148H1	22	257
161	5405506H1	2604	2840	167	5840353H1	19	294	167	4222393H1	23	304
161	4314575H1	2614	2895	167	3055393H1	1	77	167	4223817H1	22	336
161	1289406H1	2619	2877	167	4528621H1	3	250	167	5843163H1	20	289
161	g1190424	2629	2887	167	482608H1	28	255	167	3657588H1	23	218
161	g1071637	2650	2858	167	3477717H1	3	119	167	4721051H1	25	271
161	4773458H1	2681	2887	167	2472906H1	3	240	167	4790883H1	21	278
161	4756793H1	2681	2826	167	2444808H1	4	234	167	5377733H1	24	280
161	4341024H1	2694	2874	167	3167552H1	6	74	167	g1638522	24	353
161	4298642H1	2694	2887	167	2615139H1	9	226	167	3596245H1	25	318
161	1596024H1	2694	2887	167	3199504H1	13	106	167	781633H1	25	270
161	552947H1	2699	2887	167	2455307H1	10	197	167	5374490H1	26	286
161	5371715H1	2713	2886	167	2428111H1	11	248	167	2161371H1	31	277
161	5447990H2	2737	2906	167	3458104H1	12	270	167	3597248H1	32	267
161	667160H1	2739	2887	167	3596416H1	12	327	167	2934534H1	36	172
161	668331H1	2789	2883	167	2453021H1	12	213	167	4386768H1	37	326
161	5657894H1	2793	2887	167	4298232H1	12	271	167	3399780H1	126	191
162	6127609T8	1	306	167	g1956274	35	272	167	6399947H1	126	272
162	6959915H1	108	561	167	g1880655	12	256	167	6897251H1	166	634
163	5914133H1	1	282	167	3458004H1	12	279	167	4431669H2	173	312
163	5914133F6	1	630	167	5694361H1	12	286	167	g1779781	330	692
163	5321108F9	1	537	167	g2026069	15	306	167	g826488	330	717
163	5914133F8	1	391	167	477069H1	15	278	167	4794163H1	346	595
163	5914133T6	52	609	167	1918196H1	15	293	167	4880181H1	349	609

167	g889083	366	638	172	5908301H1	1	311	180	4290049F6	1	353
167	6819761J1	396	1013	172	6271267H2	24	492	180	4290049H1	1	124
167	g916666	408	513	172	5908301T9	248	586	180	5493752H1	172	444
167	4249123H1	452	539	173	5907939T9	1	517	181	6729842H1	1	412
167	6481276H1	584	800	173	5907939F8	1	551	181	5401350H1	1	105
167	4191747H1	624	709	173	5907939H1	1	310	181	6057617H1	56	643
167	638004H1	648	753	173	6271008H2	10	483	181	5401350T9	82	666
167	1452178H1	676	753	174	5912415F8	1	376	181	g3214092	406	782
167	g2615681	692	753	174	5912415H1	1	299	181	3524102H1	479	779
167	g1665347	697	753	174	5912415F6	12	565	182	7030475H1	1	524
167	g2558364	697	753	174	5912415T9	66	535	182	7030327H1	3	376
168	6795278H1	184	698	175	4119207F6	1	336	183	5271230H1	1485	1748
168	6796542H1	1	546	175	4119207T6	1	336	183	240641H1	1485	1657
168	6796380H1	8	563	175	4119207H1	1	175	183	2948213H1	1486	1768
168	6795463H1	8	523	176	5905477F6	1	564	183	g1291432	1492	1896
168	3941984H1	26	315	176	5905477H1	1	271	183	353018H1	1496	1708
168	g1260435	112	287	176	5905477T9	411	932	183	349497H1	1496	1680
168	6798249H1	184	635	177	5907791F8	1	360	183	g2276981	1533	1859
168	6791366H1	238	804	177	5907791H1	1	280	183	6804414J1	1537	2080
168	6790685H1	239	699	177	5907791F6	1	305	183	3499213H1	1537	1841
168	1242854H1	698	885	177	5907791T9	155	682	183	g2537462	1542	1981
169	2904954T6	1	522	177	5907791T6	248	733	183	1551014H1	1549	1763
169	4739603H1	11	303	178	4770137H1	1	144	183	808076H1	1554	1769
169	5614905H1	213	492	178	5564253H1	2	235	183	2307944H1	1557	1762
169	g2006850	277	557	178	606101H1	1	169	183	g2222952	1559	1851
169	2008385H1	464	562	178	4650476H1	1	271	183	2804178H1	1565	1852
169	2014576T6	464	523	178	592893H1	2	130	183	6801505J1	1566	1881
169	2014576H1	464	586	178	781453H1	17	276	183	g888315	1569	1927
169	3294227T6	223	557	178	2793564H1	28	317	183	g2056786	1570	2035
169	2014576R6	464	553	178	888943H1	151	287	183	6901949H1	1	518
169	1716729H1	501	556	178	6589872H1	194	698	183	4183549H1	91	259
170	3966795F6	1	365	178	2190828T6	207	699	183	6980581H1	104	452
170	3966795H1	1	267	178	1503654H1	225	491	183	5839778H1	107	359
170	3274864H1	14	268	178	4200384H1	388	662	183	808102H1	172	406
170	3966795T6	23	659	179	5911540F8	1	460	183	4323252H1	224	469
171	3033193F6	1	272	179	5911540H1	1	250	183	g2003585	258	517
171	3033193H1	1	216	179	5911540T9	27	568	183	g900863	361	447
171	3033193T6	129	445	179	5911540T8	78	569	183	6979927H1	437	785
172	5908301F8	1	519	180	g4325750	1	103	183	582110H1	487	739

183	3323870H1	499	778	183	3697260H1	962	1179	183	5483319H1	1402	1501
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183	4779981H1	538	793	183	g1644929	1018	1179	183	5948083H1	1409	1714
183	1818066H1	552	808	183	g1880762	1046	1163	183	1996549H1	1415	1554
183	1818066F6	552	893	183	6804414H1	1065	1601	183	1996549R6	1415	1751
183	g4438745	584	797	183	1722938H1	1074	1327	183	5405753H1	1434	1586
183	5450643H1	614	851	183	5451478H1	1104	1348	183	5293687H2	1434	1680
183	7066292H1	636	1078	183	1500938H1	1108	1304	183	935623R1	1465	1989
183	3538459H1	648	855	183	5950551H1	1120	1413	183	936611H1	1465	1767
183	2115479H1	655	913	183	3376840H1	1121	1367	183	935623H1	1465	1708
183	4090518H1	669	864	183	5950519H1	1121	1320	183	3409340H1	1473	1629
183	6077621H1	686	797	183	4193013H1	1121	1438	183	1819126H1	1480	1748
183	4947628H1	702	832	183	4556920H1	1124	1261	183	1819126F6	1480	1979
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183	1699317H1	715	931	183	g1046497	1143	1455	183	g2209615	1481	1929
183	4303659H1	740	991	183	1283213H1	1202	1390	183	5879274H1	1482	1754
183	591324R6	752	1180	183	1283213F6	1202	1658	183	4570795H1	2032	2292
183	591324H1	752	975	183	4225341H1	1206	1487	183	g1046490	2044	2349
183	591174H1	752	955	183	6714888H1	1214	1778	183	1416514H1	2046	2291
183	5998787H1	772	1179	183	1346996H1	1259	1485	183	4193606H1	2048	2324
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183	2972664H2	782	1078	183	g2107139	1290	1761	183	2222539H1	2060	2298
183	1718469H1	795	1006	183	4720048H1	1308	1583	183	2222539F6	2060	2338
183	1718480H1	795	998	183	1720725H1	1328	1538	183	2222539T6	2061	2300
183	3270027H1	802	1059	183	1722139H1	1328	1553	183	5341229H1	2066	2265
183	927251R1	806	1419	183	5970873H1	1334	1887	183	2659133H1	2067	2280
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183	5371617H1	860	1101	183	004844H1	1386	1669	183	2574115H1	2106	2337
183	3784982H1	895	1195	183	6313521H1	1386	1950	183	2273027H1	2107	2310
183	6980693H1	897	1257	183	2427675H1	1392	1622	183	g4899838	2119	2346
183	4770011H1	901	1166	183	5478565H1	1401	1608	183	g3051313	2128	2338
183	1695218H1	903	1119	183	5480821H1	1402	1635	183	3142955H1	2158	2340

183	2878176H1	2164	2339	183	3246162H1	1797	2036	183	953800T1	2000	2301
183	3271618H1	2181	2337	183	1344364H1	1798	2013	183	g895530	2006	2338
183	g2958193	2190	2340	183	1344388H1	1798	2036	183	953800H1	2012	2296
183	2247168H1	2197	2344	183	5928305H1	1801	2111	183	953800R1	2012	2338
183	2246357H1	2225	2339	183	683816H1	1805	2040	183	4977128H1	2022	2281
183	2247256H1	2254	2339	183	5451469H1	1808	2048	183	g1046498	2026	2338
183	3812939H1	2274	2338	183	2402750H1	1811	1919	184	3497339T6	1	492
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183	6818666H1	1579	1827	183	2963989H1	1819	2096	184	2570554H1	239	484
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183	g4874675	1588	2033	183	1819126T6	1843	2298	184	1400373F6	474	1007
183	5661656H1	1589	1849	183	1484988H1	1847	2095	184	1400373H1	474	733
183	6868292H1	1591	2060	183	2106229T6	1851	2298	184	g2167556	488	897
183	3706916H1	1605	1904	183	g4762523	1859	2033	184	6389638H1	542	823
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183	2424009H1	1649	1907	183	1964264H1	1872	2033	184	6344688H1	606	888
183	1470490H1	1663	1849	183	1964264R6	1872	2040	184	6328470H1	664	1018
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183	3245252H1	1669	1936	183	g2945963	1876	2340	184	6338470H1	793	1018
183	4781187H1	1671	1939	183	4696529H1	1885	2095	184	3784456H1	876	1200
183	g1688385	1690	2024	183	g4564438	1886	2339	184	3781165H1	943	1049
183	1413737H1	1712	1978	183	g3741810	1886	2341	184	g733951	1031	1403
183	g2279273	1737	2033	183	2913361H1	1889	1977	184	g734304	1149	1333
183	g1146486	1741	1926	183	6862628H1	1894	2033	184	3554877H1	1	294
183	g1644873	1745	2038	183	g3742173	1905	2340	185	4111213H1	1	243
183	g888316	1749	2033	183	g4083420	1910	2338	185	5624259R8	1	350
183	099594H1	1754	1972	183	g3678703	1912	2342	185	3139315H1	126	427
183	3996904H1	1754	1913	183	6327536H1	1912	2340	185	5545642H1	130	363
183	3916961H1	1759	2048	183	g2910125	1916	2341	185	3141553H1	139	412
183	3917411H1	1759	2036	183	g3308355	1919	2342	185	g564381	139	369
183	6801505H1	1759	2125	183	2556534H1	1933	2178	185	987690H1	154	424
183	910557H1	1785	1850	183	g3922126	1935	2338	185	4833664H1	169	319
183	2921975H1	1788	2061	183	g4083267	1936	2338	185	116132H1	214	403
183	5003193H1	1790	2060	183	g4083582	1951	2338	185	3691576H1	244	399
183	g1442965	1791	2033	183	g1646486	1958	2340	185	1667502F6	265	644
183	5018209H1	1797	1957	183	g2902884	1998	2339	185	3283023H1	267	528

185	3959293H2	290	564	189	70395867D1	1	542	190	g816766	4165	4460
185	2675859H1	347	596	189	2525961F6	4	452	190	3041690H1	4157	4451
185	2676108H1	347	594	189	2525961H1	5	246	190	977648R1	4063	4431
185	2676122H1	347	591	189	70395557D1	40	642	190	g1193159	4182	4458
185	5301231H1	387	569	189	70397658D1	59	589	190	977648T1	4063	4415
185	1598950H1	387	567	189	6560586H1	67	616	190	977648H1	4063	4386
185	2352332H1	456	626	189	70396453D1	72	478	190	978306H1	4063	4319
185	5038795H1	459	689	189	70396167D1	119	654	190	g2051455	4184	4460
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185	3252993H1	464	711	189	70396121D1	192	481	190	g3886427	4204	4347
185	1742040H1	490	721	189	g1357976	238	526	190	1381539H1	4210	4457
185	4592168H1	498	648	189	2525961T6	256	680	190	552969H1	4210	4458
185	g29244	1	255	189	70396053D1	260	679	190	2760603H1	4210	4453
185	2635196H1	1	249	189	70396654D1	278	768	190	g3647431	4212	4454
186	6428735H1	867	1184	189	70395799D1	279	680	190	1917113H1	4245	4457
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187	2525961H1	2	243	189	70397446D1	279	676	190	g892648	4259	4458
187	6560586H1	64	613	189	70397464D1	292	676	190	g2229339	4272	4456
187	g1357976	235	523	189	70394880D1	293	746	190	1942308H1	4292	4461
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188	5325512H1	23	291	190	2402420H1	4083	4349	190	2819748T6	3815	4417
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188	7289635H1	196	762	190	2791792R6	4108	4475	190	6735779H1	3822	4433
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190	1805536H1	3212	3479	190	g2026718	299	586	190	70858582V1	1366	2008
190	4160048H1	3214	3272	190	7245164H1	302	823	190	71224853V1	1396	1993
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190	5585444H1	3235	3448	190	5510632R6	364	855	190	71226033V1	1421	2078
190	6269078H1	3252	3752	190	4760384H1	491	575	190	7359924H1	1437	2064
190	127072H1	3265	3456	190	4760384F6	491	961	190	70861230V1	1450	2100
190	7290084H1	3269	3683	190	1305496F6	798	1267	190	71226687V1	1457	2020
190	7011256H1	3273	3657	190	70145686V1	798	1046	190	70864162V1	1463	2044

190	70855640V1	1491	2187	190	70855603V1	2033	2704	191	1965957R6	512	843
190	71225393V1	1507	2076	190	70858486V1	2050	2280	191	6742448H1	398	474
190	70858387V1	1539	2062	190	70858723V1	2050	2280	191	6843692H1	413	692
190	70855984V1	1520	1993	190	70861733V1	2048	2785	191	93433649	563	1009
190	70857482V1	1520	2169	190	70855437V1	2059	2683	191	6883312H1	565	1039
190	70855452V1	1526	2133	190	70858374V1	2060	2596	191	70747238V1	585	712
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190	70861119V1	1548	2117	190	71228093V1	2070	2326	191	2301872H1	512	686
190	71225746V1	1558	2147	190	71227893V1	2070	2351	191	6202014H1	534	931
190	70856590V1	1568	2109	190	71227986V1	2072	2687	191	94113683	562	1004
190	4030718F6	1573	1963	190	71225556V1	2086	2744	191	70749633V1	562	749
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190	70856815V1	1611	2119	190	70855127V1	2110	2729	191	5769401H1	682	1308
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190	70863689V1	1722	2354	191	71134281V1	928	1489	191	2106710T6	1204	1448
190	6412316H1	1730	1956	191	71130579V1	1003	1490	191	861904H1	1343	1488
190	70857818V1	1740	2409	191	71129556V1	1016	1488	191	2709045F6	1441	1798
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190	70864783V1	1755	2382	191	93427925	591	1011	192	7345973H1	862	1457
190	70863459V1	1752	2053	191	71133255V1	605	931	192	7322101H1	921	1460
190	70858650V1	1822	2413	191	70643569V1	613	810	192	6850665H1	1366	1818
190	6911209J1	1830	2415	191	70639731V1	613	829	192	2654475F6	826	1310
190	70146804V1	1869	2439	191	71133577V1	653	1365	192	2654475H1	826	1121
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190	70863645V1	1877	2491	191	968289H1	143	416	192	1787542H1	2005	2223
190	71225144V1	1889	2474	191	7212584H1	176	467	192	6930205H1	2259	2725
190	71224913V1	1929	2422	191	94735242	212	473	192	4020229H1	1952	2228
190	70146682V1	1936	2394	191	94533052	255	473	192	6894957J1	1752	2259
190	060994H1	1936	2131	191	2705278H1	1	210	192	4152694H1	1653	1911
190	70861917V1	1973	2563	191	2427258H1	8	163	192	3246645H1	1698	1866
190	2791792F6	2000	2315	191	991952H1	127	401	192	94901556	1740	2205
190	2791792H1	2000	2297	191	3070166H1	399	700	192	2740754F6	2311	2784
190	70857602V1	2001	2402	191	71131960V1	399	833	192	2740754H1	2311	2564
190	71225364V1	2013	2660	191	71130961V1	406	930	192	1834220H1	2425	2687
190	70858585V1	2015	2639	191	71133228V1	779	1472	192	4941620H1	2432	2695
190	70854964V1	2015	2644	191	2301872R6	503	813	192	4941983H1	2432	2711
190	70860984V1	2016	2573	191	91263124	505	671	192	2072075H1	2522	2772

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192	4136158H1	3193	3478	196	g4149369	2649	2925	196	2741917H1	5	251
192	2654475T6	3195	3653	196	1575363H1	2664	2888	196	6991627H1	203	727
192	3732858T6	3217	3639	196	1575363F6	2664	2917	196	1434004H1	241	528
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192	856846H1	3327	3543	196	g788188	2690	2909	196	g2880882	276	409
192	g4076116	3329	3683	196	g1482370	2691	2910	196	349280H1	375	614
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192	g5544790	141	538	196	2006905H1	2712	2899	196	g698683	403	727
192	7106835H1	489	657	196	806183H1	2716	2867	196	6916554H1	603	1106
192	2313806H1	3487	3683	196	g842117	2730	2907	196	6719552H1	659	1266
192	2841392H1	3499	3686	196	g3742246	2738	2909	196	4384945H1	704	965
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192	666777H1	3347	3550	196	4884359H1	2771	3033	196	g1885551	910	1284
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193	5911845T6	1	432	196	2343515F6	2792	2906	196	037774H1	1083	1306
193	5911845F8	1	588	196	g788214	2821	2899	196	g1717439	1121	1557
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193	5911845H1	1	254	196	5119623H1	2995	3277	196	4840554F6	1189	1633
193	5911845T8	22	443	196	3369954H1	3012	3294	196	3279387H1	1202	1456
194	5910555F8	1	587	196	5346406H1	3028	3177	196	5373713H1	1216	1468
194	5910555T8	85	489	196	2675533H1	3208	3394	196	4145650H1	1246	1331
194	5910555F6	1	640	196	3077845H1	3217	3451	196	4916687H1	1293	1450

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196	4700744H1	1373	1635	196	4324610H1	2150	2401	197	g1162029	1	146
196	6812975J1	1426	2021	196	4266784H2	2156	2439	197	g1648409	1	322
196	6484322H1	1438	1623	196	4834124H1	2174	2358	197	5833130H1	1	266
196	1912429H1	1473	1693	196	g1783896	2175	2516	197	6151063H1	53	353
196	g1440342	1498	1770	196	g2207390	2175	2518	198	6764201J1	1	585
196	g4524899	1511	1896	196	908344R2	2180	2516	198	986476R6	5	479
196	g768901	1513	1820	196	908344H1	2180	2285	198	986476H1	5	303
196	g571029	1512	1840	196	4748325H1	2199	2467	198	4180212H1	8	260
196	g880779	1513	1841	196	4746557H1	2200	2439	198	g3933038	75	566
196	g832097	1513	1884	196	g1859648	2231	2520	198	4029248H1	122	377
196	4623266H1	1566	1825	196	5565332H1	2244	2501	198	4029227H1	122	370
196	3519427H1	1590	1955	196	g778446	2320	2632	199	g4196260	1	320
196	3680478H1	1608	1840	196	g831074	2319	2636	199	g2218495	1	313
196	6332293H1	1668	2034	196	g2053046	2330	2794	199	3685061H1	1	293
196	4832174H1	1735	1965	196	3031124H1	2333	2644	199	6821713J1	1	518
196	g1329683	1739	2321	196	2277831H1	2391	2660	199	5372078H1	25	226
196	g787502	1779	2009	196	2277831R6	2391	2516	199	4205860H1	169	437
196	g864605	1779	2053	196	g2552923	2437	2906	199	4205654H1	169	416
196	g787457	1779	2024	196	g832055	2523	2923	200	6868907H1	1	542
196	g841915	1778	2047	196	g1329627	2545	2922	200	1543453R6	24	440
196	5589418H1	1784	2030	196	g4990052	2547	2834	200	1543453H1	24	217
196	5071250H1	1803	2062	196	g4311136	2547	2993	200	5055563H1	38	307
196	3865563H1	1843	2217	196	g4988520	2547	3007	200	5055563F9	38	607
196	1955962H1	1894	2151	196	g4740301	2547	2833	200	4778273F8	126	752
196	4750673H2	1945	2009	196	g3422370	2548	2923	200	4778273H1	126	397
196	442154H1	1966	2272	196	g4289096	2548	2904	200	2724784H1	137	372
196	261197H1	1965	2281	196	g3431513	2549	2876	200	2925738H1	339	605
196	444335H1	1966	2225	196	000140H1	2555	2911	200	4778273T9	559	1136
196	4515301H1	2028	2284	196	2543809H1	2555	2770	200	1543453T6	610	1221
196	3780534H1	2055	2353	196	2435619H1	2555	2713	200	1921258H1	832	1046
196	2191782F6	2063	2373	196	g5113593	2555	2917	200	1921258F6	833	1233
196	2191782H1	2063	2304	196	g4189560	2555	2877	201	g4664146	1	478
196	3735668H1	2072	2331	196	g3108551	2555	2916	201	g6140998	2	458
196	3038357H1	2117	2391	196	g1476749	2557	2878	201	g6144190	2	422
196	6812975H1	2124	2462	196	g5112875	2555	2909	201	g6139959	2	400
196	4633908H1	2138	2408	196	g4565709	2555	2921	201	6764092J1	10	589

201	g2910438	59	537	203	6183568H1	1031	1322	203	5803566H1	1165	1465
201	4570842H1	67	315	203	6183681H1	1031	1308	203	6040276H1	1169	1782
201	4921180H1	69	331	203	71001091V1	1050	1610	203	5975156H1	1169	1687
201	5955592H1	86	596	203	6217476H1	1061	1617	203	5973594H1	1169	1675
201	g2904695	89	474	203	6148902H1	1068	1615	203	5995226H1	1169	1499
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201	6982639H1	384	913	203	5806522H1	1090	1418	203	6078894H1	1169	1465
201	1570643T6	422	944	203	6146547H1	1093	1723	203	5825616H1	1173	1541
201	g5232046	430	870	203	7220801H1	1094	1676	203	5921387T8	1177	1760
201	7377338H1	537	1127	203	6132328H1	1094	1386	203	6042666H1	1187	1479
201	1570643F6	661	972	203	5995785H1	1095	1395	203	7185125H1	1194	1778
201	3119826H1	732	1002	203	7322507H1	1099	1470	203	5993778H1	1197	1463
201	6097252H1	753	954	203	6113383H1	1100	1461	203	5993775H1	1193	1513
201	1570316H1	810	972	203	5975102H1	1102	1685	203	6168554H1	1198	1502
201	1570643H1	814	972	203	6148050H1	1102	1633	203	60047572D4	1199	1757
202	5913683H1	1	281	203	5977862H1	1102	1618	203	5824125H1	1200	1655
202	5320619F9	1	372	203	6038505H1	1104	1743	203	5994991H1	1200	1515
202	5913683F6	4	358	203	7080569H1	1103	1454	203	6112929H1	1204	1502
202	5321234F9	6	484	203	6116490H1	1102	1394	203	7330358H1	1219	1909
202	5914061H1	6	264	203	71360704V1	1105	1709	203	5768301H1	1220	1806
202	5914061F8	6	444	203	5892681H1	1104	1351	203	5769260H1	1218	1756
202	5913683T6	28	431	203	6164075H1	1107	1725	203	7330481H1	1221	1868
202	5913683F8	27	200	203	6181324H1	1107	1428	203	5995163H1	1233	1569
202	6269343H1	36	444	203	5770720H1	1114	1752	203	6959960H1	1236	1747
202	6269670H1	59	444	203	5797766H1	1114	1712	203	60206936V1	1239	1912
203	71035493V1	961	1616	203	5826374H1	1118	1687	203	6034683H1	1243	1803
203	5771773H1	971	1635	203	5796423H1	1114	1401	203	6169613H1	1244	1582
203	5952931H1	971	1230	203	6036824H1	1117	1719	203	4294314T9	1258	1713
203	5989634H1	968	1168	203	6034194H1	1117	1678	203	6041063H1	1248	1905
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203	5800543H1	976	1477	203	6134213H1	1117	1460	203	5952448H1	1256	1571
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203	5798865H1	1027	1604	203	6183602H1	1162	1505	203	5771870H1	1263	1827
203	6964153H1	1031	1629	203	6183689H1	1162	1461	203	6170079H1	1262	1582

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203	5824738H1	1269	1837	203	6162572H1	648	1211	203	6135068H1	757	1061
203	3390106T6	1274	1662	203	5803707H1	648	950	203	6035941H1	759	1259
203	60221621V1	1274	1677	203	6111936H1	653	955	203	6163478H1	764	1336
203	7004345H1	1284	1909	203	6111235H1	656	969	203	5968127H1	760	1255
203	60109238B1	1286	1912	203	5953419H1	659	1020	203	6178490H1	763	1018
203	7186303H1	1290	1881	203	5995735H1	659	974	203	60204862U1	771	1320
203	7025213H1	1297	1900	203	6002288H1	659	917	203	6108986H1	773	1083
203	7182261H1	1299	1807	203	6144013H1	664	1176	203	6097994H1	787	1118
203	7193486H2	1306	1929	203	6166779H1	664	1176	203	6165174H1	793	1081
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203	5970654H1	1322	1912	203	6053478J1	664	1250	203	5798369H1	797	1419
203	5770017H1	1327	1919	203	6052294J1	666	1098	203	5801102H1	797	1105
203	5802957H1	1327	1561	203	5995222H1	671	1001	203	6112951H1	797	997
203	5800770H1	1328	1601	203	5990146H1	671	994	203	6109295H1	803	1129
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203	7331927H1	1332	1935	203	5975323H1	681	1238	203	5955376H1	806	1170
203	5769517H1	1333	1918	203	7327981H2	684	1173	203	7359669H1	815	1090
203	5967562H1	1333	1856	203	6155086H1	691	1046	203	6176408H1	813	1095
203	6163438H1	1337	1896	203	6183751H1	699	1001	203	7004683H1	820	1427
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203	5789044H1	1338	1633	203	6215696H1	701	1298	203	71033163V1	820	1364
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203	6150854H1	647	996	203	6154930H1	741	1095	203	5915476H1	858	1096
203	5804474H1	647	944	203	6145090H1	747	1336	203	6163363H1	863	1499
203	6043158H1	647	922	203	7215869H1	750	1359	203	5796628H1	870	1046

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203	6172878H1	890	1197	203	4571391F8	75	628	203	7325279H1	201	812
203	6172790H1	890	1176	203	5771842H1	79	676	203	6116494H1	204	493
203	5801236H1	902	1168	203	6108158H1	79	418	203	5953527H1	210	543
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203	6130142H1	908	1245	203	6179512H1	79	393	203	6109818H1	226	448
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203	6037518H1	915	1525	203	7008218H1	79	694	203	6182368H1	242	578
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203	6133149H1	913	1026	203	60126922U1	86	662	203	6170505H1	265	596
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203	5970884H1	924	1510	203	6182051H1	91	394	203	5838053H1	296	585
203	6113366H1	923	1026	203	7272693H1	91	677	203	5796171H1	304	858
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203	5975145H1	947	1489	203	5968167H1	118	728	203	6966778H1	329	425
203	6044290H1	941	1226	203	60120598D1	108	278	203	6172080H1	328	576
203	5768644H1	957	1532	203	6147691H1	122	616	203	7182401H1	354	954
203	5971326H1	948	1554	203	6115311H1	133	425	203	7269240H1	357	994
203	6110988H1	946	1173	203	5975411H1	137	630	203	6029089H1	360	667
203	6099321H1	948	1236	203	5915392H1	144	416	203	6100553H1	367	674
203	6173091H1	62	390	203	6130193H1	148	465	203	5825841H1	387	1002
203	6036155H1	65	613	203	7328558H1	147	830	203	7214023H1	396	923
203	6180780H1	65	387	203	5996192H1	166	467	203	6109604H1	393	729
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203	7024381H1	67	430	203	6109928H1	179	509	203	6136677H1	423	744

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203	6053478H1	452	1086	203	5973147H1	35	622	203	5989784H1	39	328
203	6109628H1	453	711	203	6735535H1	39	221	203	6135227H1	39	339
203	6044864J1	455	1098	203	7279008H1	39	531	203	6028575H1	39	267
203	7180879H1	457	1063	203	60264017D1	32	569	203	5914782H1	57	278
203	5797850H1	461	930	203	7079629H2	44	358	203	7359725H1	39	685
203	6115841H1	461	756	203	5972573H1	41	686	203	6084122H1	40	673
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203	6731970H1	474	1055	203	6784013H1	35	645	203	7358734H1	39	682
203	6108268H1	478	793	203	7083178H1	36	637	203	7181284H1	39	557
203	6115462H1	491	718	203	7182216H1	36	592	203	60264004D1	39	552
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203	7360854H1	525	694	203	7261837H1	36	644	203	6174304H1	39	387
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203	6109032H1	534	897	203	6170740H1	37	340	203	6132067H1	39	350
203	5892176H1	534	868	203	7262607H1	39	648	203	6099630H1	39	322
203	5953990H1	534	804	203	7220667H1	38	636	203	6134055H1	62	379
203	5831262H1	534	726	203	7182859H1	39	616	203	5994380H1	42	384
203	5955474H1	543	886	203	6099372H1	39	324	203	6098224H1	42	362
203	5955458H1	543	888	203	7260783H1	39	675	203	6132993H1	42	337
203	6111439H1	544	769	203	7316234H1	39	709	203	7213881H1	43	644
203	6111316H1	546	881	203	4625563F9	39	673	203	6081567H1	43	636
203	5797137H1	577	1094	203	7160372H1	39	643	203	6132164H1	43	385
203	6133207H1	586	897	203	7160758H1	39	615	203	6101109H1	43	377
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203	7261854H1	590	1225	203	7219614H1	39	613	203	6078760H1	43	365
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203	5824550H1	606	1060	203	7317368H1	39	420	203	5989890H1	43	350
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203	5771704H1	626	1260	203	6171834H1	39	358	203	6111434H1	43	339
203	6131361H1	624	937	203	6169216H1	39	372	203	6168435H1	43	284
203	71216557V1	628	1188	203	6131659H1	39	358	203	7268992H1	45	675
203	6141011H1	1	156	203	6177913H1	39	350	203	6081557H1	44	614
203	6108212H1	1	324	203	6109871H1	39	347	203	6110567H1	43	283
203	6177861H1	2	301	203	6132882H1	39	346	203	7272829H1	45	685

203	5992910H1	45	388	203	6034778H1	59	683	203	5990839H1	1456	1799
203	6131653H1	47	373	203	7330333H1	58	524	203	5768633H1	1459	1920
203	5796567H1	45	109	203	7184720H1	62	640	203	96229211	1555	1933
203	7263260H1	46	647	203	60104786B2	1354	1910	203	96132479	1555	1933
203	7158311H1	46	588	203	6177422H1	1353	1657	203	96030521	1555	1935
203	6097721H1	47	393	203	5824565H1	1360	1894	203	96197062	1561	1937
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203	6170754H1	47	353	203	6042367H1	1364	1661	203	96471957	1565	1928
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203	7275183H1	48	644	203	6170972H1	1365	1682	203	6030104H2	1566	1861
203	7182041H1	49	616	203	6181601H1	1365	1667	203	5805947H1	1565	1840
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203	5991415H1	48	388	203	4362784F8	1372	1927	203	96030549	1568	1928
203	6112705H1	48	373	203	5996082H1	1377	1699	203	96198562	1567	1928
203	6098861H1	48	347	203	5996003H1	1375	1666	203	6097965H1	1568	1889
203	6099983H1	48	243	203	5995203H1	1382	1721	203	6151677H1	1571	1866
203	7283631H1	49	718	203	6133437H1	1383	1700	203	5974518H1	1571	1926
203	6039521H1	49	650	203	6178414H1	1393	1699	203	96229210	1574	1933
203	5992224H1	49	375	203	6179001H1	1401	1548	203	6028702H1	1579	1813
203	6170022H1	49	387	203	5769429H1	1403	1918	203	96470581	1580	1920
203	5992273H1	49	363	203	5785086H1	1404	1722	203	96198908	1587	1928
203	6114538H1	48	348	203	5801902H1	1405	1725	203	96044672	1588	1928
203	7272102H1	50	635	203	5792833H1	1404	1477	203	96470803	1511	1930
203	6136863H1	50	389	203	5949501H1	1415	1784	203	96027906	1513	1934
203	6085163H1	68	331	203	96228962	1428	1937	203	96074740	1524	1928
203	6115314H1	50	306	203	96228709	1432	1938	203	96026164	1523	1928
203	7270954H1	51	687	203	96300732	1431	1938	203	96133768	1528	1932
203	7190289H2	51	619	203	60126922B1	1434	1887	203	6179968H1	1526	1784
203	4624253F8	54	733	203	6173439H1	1434	1747	203	5991468H1	1528	1863
203	6037863H1	54	590	203	5769804H1	1434	1920	203	5786634H1	1530	1832
203	5973394H1	54	630	203	96301637	1436	1938	203	5786701H1	1530	1715
203	6168342H1	54	412	203	96302015	1437	1936	203	5786734H1	1532	1652
203	6733426H1	73	419	203	5953730H1	1440	1530	203	96047869	1534	1921
203	5770643H1	55	644	203	5770049H1	1440	1927	203	6176458H1	1547	1825
203	6733166H1	54	647	203	7082145H1	1440	1907	203	5816770H1	1550	1883
203	7321688H1	58	593	203	96301063	1443	1936	203	5817968H1	1550	1746
203	6038935H1	58	635	203	6133907H1	1444	1778	203	96471065	1550	1928
203	6134077H1	56	399	203	96039672	1445	1932	203	5817174H1	1550	1832
203	7185189H1	56	643	203	96301561	1453	1938	203	96471718	1552	1937

203	g6464005	1552	1937	203	g6131743	1480	1935	203	5946071H1	1668	1917
203	6108818H1	1554	1787	203	g6044247	1480	1928	203	5950118H1	1668	1905
203	7181625H1	1553	1920	203	6034847H1	1482	1911	203	6157445H1	1668	1850
203	g6043984	1488	1938	203	g6117455	1481	1933	203	5946973H1	1668	1920
203	g6476041	1488	1930	203	g6464482	1481	1928	203	5767935H1	1670	1920
203	g6075390	1489	1937	203	g6036141	1481	1928	203	5767973H1	1670	1912
203	g6028910	1490	1937	203	g6047637	1481	1928	203	5947508H1	1672	1934
203	g6073495	1490	1930	203	g6117632	1482	1930	203	5790266H1	1674	1920
203	g6401184	1490	1930	203	g6463078	1482	1936	203	g6400408	1676	1938
203	g6073672	1490	1937	203	g6035703	1480	1936	203	7338126H1	1680	1932
203	g6036791	1489	1928	203	g6076131	1482	1932	203	7350525H1	1686	1936
203	g6026147	1492	1935	203	g6196780	1482	1932	203	g6398110	1686	1938
203	g6036640	1492	1920	203	g6031028	1483	1930	203	g6402278	1686	1938
203	6157091H1	1492	1813	203	g6464375	1484	1928	203	6157246H1	1686	1827
203	g6033899	1493	1928	203	g6043231	1484	1929	203	g6197883	1687	1928
203	g6198865	1493	1938	203	g6117300	1485	1933	203	g6046641	1691	1928
203	6219812H2	1500	1886	203	g6200216	1484	1938	203	g6400764	1692	1928
203	6192668H1	1501	1814	203	g6040103	1485	1932	203	g6076220	1693	1928
203	5768793H1	1505	1920	203	g6399002	1485	1926	203	5794559H1	1695	1926
203	6191666H1	1501	1804	203	g6029144	1486	1934	203	g6033633	1695	1926
203	g6473184	1502	1934	203	g6043655	1487	1937	203	5787563H1	1695	1925
203	g6476087	1502	1928	203	g6196472	1489	1934	203	g6117328	1708	1937
203	5993364H1	1503	1813	203	g6034277	1815	1920	203	g6044253	1710	1929
203	g6438645	1503	1928	203	g6117025	1815	1920	203	g6398246	1721	1928
203	g6076043	1504	1928	203	g6199037	1815	1920	203	g6131945	1465	1929
203	6043152H1	1519	1787	203	g6198912	1823	1928	203	6181714H1	1466	1806
203	g6472040	1509	1937	203	g6473327	1833	1928	203	g6048081	1466	1928
203	6179110H1	1511	1806	203	g6463164	1841	1930	203	g6047077	1466	1932
203	g6400409	1476	1929	203	5920960H1	1844	1925	203	g6132102	1468	1929
203	g6036079	1476	1928	203	g6198784	1856	1926	203	g6398647	1468	1929
203	g6086332	1477	1934	203	5787770H1	1722	1981	203	g6400444	1468	1929
203	g6199417	1477	1933	203	5789227H1	1722	1927	203	g6086266	1468	1928
203	g6044232	1478	1936	203	g6074506	1728	1938	203	g6132321	1469	1928
203	g6029466	1478	1935	203	6166065H1	1741	1956	203	g6132239	1470	1930
203	g6439518	1479	1934	203	g6029762	1746	1876	203	g6074116	1468	1928
203	g6462956	1479	1934	203	5786021H1	1754	1926	203	5990574H1	1470	1804
203	g6439604	1480	1937	203	g6438899	1763	1928	203	g6400620	1470	1930
203	g6196631	1480	1938	203	5992585H1	1769	1920	203	g6074889	1470	1928
203	g6439647	1479	1930	203	6158235H1	1865	1940	203	g6473566	1469	1928

203	g6400824	1472	1936	204	1426502T6	979	1248	204	1299074H1	161	401
203	g6132812	1472	1930	204	2825904H1	984	1241	204	6824091H1	156	422
203	g6400805	1472	1938	204	6825511H1	594	1115	204	3698810H1	13	304
203	g6400717	1472	1928	204	6827717H1	676	1155	204	1751671H1	621	843
203	g6030907	1473	1928	204	6828967J1	813	1145	204	g1616031	448	783
203	g6047234	1474	1930	204	6825030H1	823	1116	204	6824349J1	147	796
203	7180844H1	1459	1928	204	6825030J1	822	1115	204	4516155H1	541	775
203	g6035938	1464	1916	204	1470306H1	921	1114	204	4672305H1	94	298
203	g6439666	1475	1928	204	6827717J1	434	1076	204	6829925H1	1303	1619
203	g6036991	1475	1928	204	1945447H1	808	1051	204	1426502H1	1438	1679
203	g6047968	1475	1930	204	5870352H1	729	1007	204	6829925J1	1303	1618
203	g6036801	1476	1937	204	6826536J1	1157	1756	204	6824269J1	875	1484
203	g6196212	1600	1928	204	6822128J1	829	984	204	6824288J1	805	1385
203	6177877H1	1603	1888	204	6826536H1	1143	1742	204	6825511J1	786	1392
203	g6039199	1605	1928	204	g3056033	1065	1305	204	g5856181	886	1309
203	g6438679	1604	1920	204	g5837846	816	1289	204	g824615	1112	1275
203	g6400557	1611	1928	204	g2986193	1027	1297	204	6802929J1	833	1289
203	5817262H1	1615	1904	204	6831310H1	845	1278	204	g5127144	929	1299
203	5817362H1	1615	1906	204	1631532H1	1227	1297	204	g2344128	1075	1315
203	5820474H1	1615	1915	204	g3150672	914	1287	204	6458046H1	929	1285
203	6102129H1	1619	1928	204	g2236974	899	1287	204	g2397910	1064	1302
203	g6074067	1653	1932	204	g4889379	1021	1302	204	g2278427	1157	1301
203	g6132060	1656	1935	204	2255054R6	393	777	204	g3058045	823	1284
203	3775108F9	1656	1912	204	6498130H1	312	777	204	6820991H1	747	1284
203	g6198405	1665	1929	204	2255054H1	549	777	204	2255054T6	705	1267
203	6178153H1	1665	1928	204	6826447H1	357	752	204	6822128H1	514	951
203	g6045038	1666	1911	204	6826447J1	357	752	204	6802929H1	535	920
203	5770026H1	1668	1946	204	1579339H1	502	713	204	6825384H1	424	928
203	5792284H1	1668	1920	204	4443559H1	474	613	204	6824288H1	498	991
203	g6028253	1588	1930	204	2042892H1	315	568	204	6824091J1	335	879
203	g6475978	1593	1932	204	3358238H1	329	566	204	3512547H1	1455	1695
203	6175948H1	1593	1891	204	4750087H1	185	453	204	6804257H1	1157	1691
203	g6132639	1594	1928	204	6824369H1	386	880	204	3700061H1	1404	1683
203	g6463340	1597	1928	204	6824349H1	350	851	204	1426502F6	1335	1679
203	g6046756	1596	1928	204	2184748H1	179	463	204	755545H1	1439	1679
204	6844571H1	664	1221	204	6756704J1	235	808	204	6498381H1	1158	1726
204	1299074T6	715	1264	204	2184748F6	1	463	205	1858538T6	1354	1583
204	1751671T6	860	1258	204	6756496H1	234	432	205	1810993T6	1360	1586
204	6941680H1	769	1245	204	6756704H1	255	432	205	1810792H1	1281	1506

205	2625825H1	1367	1595	206	2487001F6	672	1061	207	g5232991	277	675
205	2228636T6	1368	1586	206	2487001H1	672	829	207	g3090059	202	676
205	1724417T6	1386	1586	206	4980831H1	862	1147	207	g3751723	261	676
205	2689550T6	1333	1586	206	2620607H1	875	1129	207	g4299176	248	676
205	2689550F6	1	511	206	3873170H1	876	1028	207	g5630519	273	675
205	2689550H1	1	265	206	4575663H1	912	1190	207	5743674T7	354	545
205	6476804H1	3	563	206	6155913H1	953	1276	207	5743674H1	1	297
205	1545750H1	17	163	206	4543658H1	1005	1249	207	4127943H1	1	246
205	g2244465	68	341	206	5064775H1	1104	1345	207	5743674R7	1	183
205	1494653H1	351	402	206	g1014062	1144	1418	208	1952854T6	1	565
205	1858538H1	400	682	206	6249620H1	1173	1668	209	2917244T6	1	567
205	2218749F6	474	916	206	2881132F6	1223	1489	209	2917244H1	1	156
205	2228636F6	474	908	206	2881132H1	1223	1496	209	2917244F6	1	567
205	2228636H1	474	714	206	g1949025	1228	1546	210	5507875H1	1	219
205	1724288H1	642	852	206	4552262H1	1238	1500	210	5507875F6	1	393
205	1724417H1	642	852	206	950155H1	1278	1523	210	3510289H1	1	276
205	1724417F6	642	998	206	1705026H1	1316	1527	210	6985373H1	20	382
205	1724064H1	642	841	206	4398180H1	1331	1571	210	6985355H1	20	540
205	1724064F6	642	1096	206	3179825T6	1418	1909	210	4715879H1	20	238
205	6549336H1	1002	1419	206	2487001T6	1441	2006	210	645341H1	21	220
205	4822845H1	1024	1261	206	6097383H1	1482	1798	210	645341R6	21	432
205	1810993F6	1281	1558	206	g2251371	1596	2047	210	4551228H1	20	212
205	1810993H1	1281	1518	206	g4072810	1631	2050	210	3182009H1	24	326
206	6045641H1	1	364	206	g3889774	1700	2050	210	3182022H1	24	321
206	6045641J1	1	364	206	g4087488	1749	2056	210	5156573H1	27	272
206	3179825H1	120	424	206	g1014063	1798	2030	210	4800844H1	28	277
206	3179825F6	120	672	206	2881132T6	1809	2005	210	5864355H1	31	324
206	4758644H1	141	409	206	g1195443	1885	2047	210	6219781H1	32	251
206	2966833H1	222	484	206	g2155148	1942	2053	210	3375196H1	35	280
206	5372448H1	327	481	207	6803514H1	503	984	210	6306192H1	38	512
206	2686267H1	359	619	207	6803514J1	242	823	210	g575091	38	316
206	3722865H1	425	558	207	6286909H2	316	799	210	2924014F6	38	385
206	5036810H1	463	731	207	g4568721	260	685	210	2924014H1	38	247
206	4164428H1	519	787	207	g3742689	209	684	210	2825826H1	56	355
206	3799675H1	563	731	207	g5365250	586	679	210	3695750H1	62	182
206	4708839H1	568	851	207	g5676607	451	679	210	941644R6	66	275
206	2952970H1	602	865	207	g4111591	263	679	210	5310424H1	66	282
206	3561988H1	633	928	207	g2539496	299	678	210	3580982H1	36	340
206	856538H1	642	846	207	g5769156	224	677	210	941644R1	66	365

210	5967756H1	70	570
210	9831059	85	445
210	5727850H1	131	666
210	2053435H1	218	446
210	94682505	234	685
210	5507875R6	308	769
210	6874493H1	354	971
210	95109730	543	998
210	91391905	560	858
211	6409981H1	1	486

TABLE 5		
SEQ ID NO:	Template ID	Tissue Distribution
1	LG:1040582.1:2000FEB18	Liver - 41%, Pancreas - 34%, Cardiovascular System - 14%
2	LG:453570.1:2000FEB18	Nervous System - 100%
3	LG:408751.3:2000FEB18	Sense Organs - 63%, Nervous System - 22%
4	LI:090574.1:2000FEB01	Nervous System - 46%, Unclassified/Mixed - 36%
5	LI:229932.2:2000FEB01	Musculoskeletal System - 80%
6	LI:332176.1:2000FEB01	Urinary Tract - 95%
7	LI:403248.2:2000FEB01	Respiratory System - 60%, Hemic and Immune System - 40%
8	LG:220992.1:2000MAY19	Embryonic Structures - 17%, Male Genitalia - 12%
9	LG:1094571.1:2000MAY19	Liver - 19%, Embryonic Structures - 16%, Cardiovascular System - 14%
10	LI:350754.4:2000MAY01	Skin - 47%, Stomatognathic System - 27%, Sense Organs - 14%
11	LI:255828.29:2000MAY01	Musculoskeletal System - 100%
12	LI:1190263.1:2000MAY01	Urinary Tract - 80%, Urinary Tract - 15%
13	LG:270916.2:2000FEB18	Female Genitalia - 100%
14	LG:999414.3:2000FEB18	Embryonic Structures - 30%, Urinary Tract - 13%, Digestive System - 11%, Musculoskeletal System - 11%
15	LG:429446.1:2000FEB18	Urinary Tract - 80%, Hemic and Immune System - 20%
16	LI:057229.1:2000FEB01	Male Genitalia - 71%, Hemic and Immune System - 29%
17	LI:351965.1:2000FEB01	Unclassified/Mixed - 53%, Male Genitalia - 12%
18	LG:068682.1:2000FEB18	Unclassified/Mixed - 49%, Male Genitalia - 27%
19	LG:242665.1:2000FEB18	Germ Cells - 47%, Female Genitalia - 13%, Male Genitalia - 12%
20	LG:241743.1:2000FEB18	Liver - 27%, Urinary Tract - 27%, Respiratory System - 14%
21	LI:034212.1:2000FEB01	Digestive System - 24%, Musculoskeletal System - 22%, Nervous System - 11%
22	LG:344886.1:2000MAY19	Germ Cells - 24%, Nervous System - 12%
23	LG:228930.1:2000MAY19	Embryonic Structures - 43%, Nervous System - 29%, Respiratory System - 14%, Hemic and Immune System - 14%
24	LG:338927.1:2000MAY19	Digestive System - 23%, Unclassified/Mixed - 21%, Embryonic Structures - 19%, Hemic and Immune System - 19%
25	LG:898771.1:2000MAY19	Pancreas - 13%, Embryonic Structures - 11%, Female Genitalia - 10%, Urinary Tract - 10%, Hemic and Immune System - 10%, Cardiovascular System - 10%
26	LI:257664.67:2000MAY01	Hemic and Immune System - 100%
27	LI:001496.2:2000MAY01	Endocrine System - 27%, Female Genitalia - 25%, Embryonic Structures - 25%
28	LI:1085273.2:2000MAY01	Digestive System - 29%, Skin - 24%, Endocrine System - 16%
29	LI:333138.2:2000MAY01	Exocrine Glands - 61%, Nervous System - 13%, Nervous System - 11%
30	LI:338927.1:2000MAY01	Embryonic Structures - 51%, Digestive System - 17%
31	LG:335558.1:2000FEB18	Endocrine System - 45%, Nervous System - 18%, Exocrine Glands - 11%

32	LG:998283.7:2000FEB18	Sense Organs - 33%, Germ Cells - 18%
33	LI:402739.1:2000FEB01	Unclassified/Mixed - 78%, Male Genitalia - 11%,
34	LI:175223.1:2000FEB01	Hemic and Immune System - 11%
		Embryonic Structures - 99%
		Endocrine System - 28%, Nervous System - 22%,
		Respiratory System - 17%, Female Genitalia - 17%,
35	LG:981076.2:2000MAY19	Hemic and Immune System - 17%
36	LI:1008973.1:2000MAY01	Nervous System - 57%, Digestive System - 41%
37	LI:1190250.1:2000MAY01	Female Genitalia - 48%, Respiratory System - 25%
		Liver - 23%, Endocrine System - 17%, Hemic and
38	LG:021371.3:2000FEB18	Immune System - 17%
39	LG:475404.1:2000FEB18	Skin - 82%
		Liver - 46%, Connective Tissue - 31%, Nervous
40	LG:979406.2:2000FEB18	System - 15%
		Embryonic Structures - 52%, Endocrine System -
41	LG:410726.1:2000FEB18	26%
		Unclassified/Mixed - 26%, Cardiovascular System -
42	LG:200005.1:2000FEB18	14%, Female Genitalia - 13%
43	LG:1076828.1:2000FEB18	Unclassified/Mixed - 69%, Urinary Tract - 25%
		Unclassified/Mixed - 63%, Musculoskeletal System -
44	LG:1076931.1:2000FEB18	20%, Urinary Tract - 11%
45	LG:1078121.1:2000FEB18	Female Genitalia - 75%, Nervous System - 25%
		Female Genitalia - 42%, Cardiovascular System -
46	LG:1079203.1:2000FEB18	33%, Hemic and Immune System - 17%
47	LG:1082586.1:2000FEB18	Respiratory System - 100%
48	LG:1082774.1:2000FEB18	Respiratory System - 50%, Female Genitalia - 50%
49	LG:1082775.1:2000FEB18	Female Genitalia - 75%, Nervous System - 25%
50	LG:1083120.1:2000FEB18	Nervous System - 100%
51	LG:1087707.1:2000FEB18	Stomatognathic System - 98%
		Embryonic Structures - 44%, Connective Tissue -
52	LG:1090915.1:2000FEB18	19%
53	LG:1094230.1:2000FEB18	Female Genitalia - 100%
		Connective Tissue - 44%, Exocrine Glands - 44%,
54	LG:474848.3:2000FEB18	Hemic and Immune System - 11%
		Nervous System - 38%, Digestive System - 38%, Male
55	LI:251656.1:2000FEB01	Genitalia - 25%
		Hemic and Immune System - 69%, Endocrine
56	LI:021371.1:2000FEB01	System - 14%
57	LI:133095.1:2000FEB01	Respiratory System - 67%, Nervous System - 13%
		Unclassified/Mixed - 30%, Respiratory System - 19%,
		Nervous System - 13%, Digestive System - 13%
58	LI:236654.2:2000FEB01	
		Unclassified/Mixed - 37%, Urinary Tract - 16%,
59	LI:200009.1:2000FEB01	Cardiovascular System - 15%
		Unclassified/Mixed - 78%, Musculoskeletal System -
60	LI:758502.1:2000FEB01	22%
		Nervous System - 56%, Skin - 27%, Connective Tissue
61	LI:344772.1:2000FEB01	- 13%
62	LI:789445.1:2000FEB01	Endocrine System - 100%
		Urinary Tract - 31%, Female Genitalia - 19%,
		Digestive System - 19%, Hemic and Immune System
63	LI:789657.1:2000FEB01	- 19%
		Exocrine Glands - 44%, Female Genitalia - 33%,
64	LI:789808.1:2000FEB01	Nervous System - 22%
65	LI:792919.1:2000FEB01	Respiratory System - 100%

66	LI:793949.1:2000FEB01	Female Genitalia - 42%, Endocrine System - 19%, Exocrine Glands - 13%
67	LI:794389.1:2000FEB01	Endocrine System - 100%
68	LI:796010.1:2000FEB01	Exocrine Glands - 100%
69	LI:796324.1:2000FEB01	Female Genitalia - 100%
70	LI:796373.1:2000FEB01	Respiratory System - 100%
71	LI:796415.1:2000FEB01	Nervous System - 100%
72	LI:798636.1:2000FEB01	Hemic and Immune System - 100%
73	LI:800045.1:2000FEB01	Female Genitalia - 60%, Male Genitalia - 40%
74	LI:800680.1:2000FEB01	Cardiovascular System - 100%
75	LI:800894.1:2000FEB01	Respiratory System - 50%, Digestive System - 50%
76	LI:801015.1:2000FEB01	Male Genitalia - 100%
77	LI:801236.1:2000FEB01	Endocrine System - 100%
78	LI:803335.1:2000FEB01	Connective Tissue - 100%
79	LI:803998.1:2000FEB01	Nervous System - 38%, Digestive System - 38%, Male Genitalia - 25%
80	LI:478757.1:2000FEB01	Digestive System - 100%
81	LI:808532.1:2000FEB01	Hemic and Immune System - 100%
82	LI:443073.1:2000FEB01	Digestive System - 100%
83	LI:479671.1:2000FEB01	Exocrine Glands - 80%, Hemic and Immune System - 20%
84	LI:810078.1:2000FEB01	Digestive System - 100%
85	LI:810224.1:2000FEB01	Digestive System - 100%
86	LI:817052.2:2000FEB01	Nervous System - 24%, Unclassified/Mixed - 18%, Exocrine Glands - 14%
87	LG:892274.1:2000MAY19	Embryonic Structures - 63%, Digestive System - 30%
88	LG:1080959.1:2000MAY19	Digestive System - 40%, Respiratory System - 30%, Hemic and Immune System - 30%
89	LG:1054900.1:2000MAY19	Digestive System - 100%
90	LG:1077357.1:2000MAY19	Nervous System - 38%, Female Genitalia - 38%, Male Genitalia - 25%
91	LG:1084051.1:2000MAY19	Pancreas - 31%, Digestive System - 22%, Hemic and Immune System - 16%
92	LG:1076853.1:2000MAY19	Female Genitalia - 23%, Unclassified/Mixed - 23%, Cardiovascular System - 18%, Exocrine Glands - 18%
93	LG:481631.10:2000MAY19	Female Genitalia - 22%, Nervous System - 17%, Exocrine Glands - 17%, Urinary Tract - 17%
94	LG:1088431.2:2000MAY19	Exocrine Glands - 67%, Cardiovascular System - 33%
95	LI:401619.10:2000MAY01	Endocrine System - 18%, Embryonic Structures - 16%, Pancreas - 15%
96	LI:1144007.1:2000MAY01	Hemic and Immune System - 27%, Female Genitalia - 13%
97	LI:331074.1:2000MAY01	Endocrine System - 28%, Sense Organs - 22%, Connective Tissue - 10%
98	LI:1170349.1:2000MAY01	Endocrine System - 91%
99	LG:335097.1:2000FEB18	Embryonic Structures - 24%, Musculoskeletal System - 19%, Nervous System - 16%
100	LG:1076451.1:2000FEB18	Nervous System - 100%
101	LI:805478.1:2000FEB01	Skin - 100%
102	LG:101269.1:2000MAY19	Endocrine System - 33%, Embryonic Structures - 33%, Urinary Tract - 30%
103	LI:331087.1:2000MAY01	Liver - 82%, Hemic and Immune System - 13%

104	LI:410188.1:2000MAY01	Cardiovascular System - 81%, Cardiovascular System - 12%
105	LI:1188288.1:2000MAY01	Nervous System - 73% Liver - 16%, Male Genitalia - 13%, Embryonic Structures - 11%
106	LI:427997.4:2000MAY01	Nervous System - 100%
107	LG:451682.1:2000FEB18	Liver - 86%, Hemic and Immune System - 14%
108	LG:1077283.1:2000FEB18	Embryonic Structures - 41%, Endocrine System - 20%, Hemic and Immune System - 13%
109	LG:481436.5:2000FEB18	Endocrine System - 43%, Urinary Tract - 36%, Respiratory System - 21%
110	LI:793701.1:2000FEB01	Germ Cells - 74%, Unclassified/Mixed - 16%
111	LI:373637.1:2000FEB01	Digestive System - 43%, Male Genitalia - 24%, Endocrine System - 24%
112	LG:239368.2:2000MAY19	Germ Cells - 66%, Unclassified/Mixed - 22%, Male Genitalia - 12%
113	LI:053826.1:2000MAY01	Nervous System - 100%
114	LI:449393.1:2000MAY01	Stomatognathic System - 13%
115	LI:1071427.96:2000MAY01	Unclassified/Mixed - 55%, Connective Tissue - 26%
116	LI:336338.8:2000MAY01	Urinary Tract - 24%, Hemic and Immune System - 24%, Respiratory System - 18%
117	LG:345527.1:2000FEB18	Connective Tissue - 73%, Female Genitalia - 27%
118	LG:1089383.1:2000FEB18	Female Genitalia - 38%, Exocrine Glands - 31%, Male Genitalia - 15%, Hemic and Immune System - 15%
119	LG:1092522.1:2000FEB18	Urinary Tract - 100%
120	LG:1093216.1:2000FEB18	Embryonic Structures - 86%, Hemic and Immune System - 14%
121	LI:270318.3:2000FEB01	Unclassified/Mixed - 34%, Hemic and Immune System - 20%, Urinary Tract - 17%
122	LI:335671.2:2000FEB01	Nervous System - 62%, Urinary Tract - 38%
123	LI:793758.1:2000FEB01	Female Genitalia - 100%
124	LI:803718.1:2000FEB01	Endocrine System - 100%
125	LI:412179.1:2000FEB01	Digestive System - 75%
126	LI:815679.1:2000FEB01	Embryonic Structures - 28%, Skin - 20%, Unclassified/Mixed - 16%
127	LI:481361.3:2000FEB01	Cardiovascular System - 33%, Endocrine System - 21%, Male Genitalia - 21%
128	LG:247388.1:2000MAY19	Endocrine System - 56%, Urinary Tract - 44%
129	LG:255789.10:2000MAY19	Endocrine System - 22%, Digestive System - 13%
130	LI:787618.1:2000MAY01	Endocrine System - 12% Sense Organs - 18%, Nervous System - 11%

139	LG:337818.2:2000FEB18	Digestive System - 34%, Liver - 17%, Female Genitalia - 11%
140	LI:337818.1:2000FEB01	Digestive System - 27%, Liver - 19%, Female Genitalia - 15%
141	LG:241577.4:2000MAY19	Pancreas - 48%, Endocrine System - 24%, Respiratory System - 14%
142	LG:344786.4:2000MAY19	Respiratory System - 67%, Digestive System - 22%, Nervous System - 11%
143	LI:414307.1:2000FEB01	Endocrine System - 44%, Unclassified/Mixed - 17%, Nervous System - 11%
144	LI:202943.2:2000FEB01	Embryonic Structures - 100%
145	LI:246194.2:2000FEB01	Germ Cells - 75%, Pancreas - 13%
146	LI:815961.1:2000FEB01	Digestive System - 99%
147	LG:120744.1:2000MAY19	Skin - 33%, Embryonic Structures - 21%, Digestive System - 21%
148	LI:757520.1:2000MAY01	Musculoskeletal System - 45%, Cardiovascular System - 26%, Skin - 24%
149	LG:160570.1:2000FEB18	Skin - 84%, Female Genitalia - 16%
150	LI:350398.3:2000FEB01	Male Genitalia - 50%, Hemic and Immune System - 50%
151	LI:221285.1:2000FEB01	Endocrine System - 42%, Nervous System - 21%
153	LI:329017.1:2000FEB01	Endocrine System - 62%, Unclassified/Mixed - 24%
154	LI:401322.1:2000FEB01	Sense Organs - 44%, Liver - 22%, Skin - 14%
155	LG:403409.1:2000MAY19	Respiratory System - 18%, Female Genitalia - 16%, Cardiovascular System - 13%
156	LG:233933.5:2000MAY19	Digestive System - 100%
157	LI:290344.1:2000MAY01	Connective Tissue - 40%, Nervous System - 19%, Embryonic Structures - 12%
158	LI:410742.1:2000MAY01	Respiratory System - 47%, Skin - 42%
159	LG:406568.1:2000MAY19	Stomatognathic System - 57%, Musculoskeletal System - 21%, Cardiovascular System - 16%
160	LI:283762.1:2000MAY01	Sense Organs - 25%
161	LI:347687.113:2000MAY01	Nervous System - 45%, Nervous System - 38%
162	LI:1146510.1:2000MAY01	Skin - 94%
163	LG:451710.1:2000FEB18	Connective Tissue - 89%, Nervous System - 11%
164	LG:455771.1:2000FEB18	Nervous System - 100%
165	LG:452089.1:2000FEB18	Nervous System - 100%
166	LG:246415.1:2000FEB18	Pancreas - 83%, Nervous System - 17%
167	LG:414144.10:2000FEB18	Cardiovascular System - 17%, Connective Tissue - 12%
168	LG:1101445.1:2000FEB18	Liver - 91%
169	LG:452134.1:2000FEB18	Hemic and Immune System - 64%, Male Genitalia - 36%
170	LI:903021.1:2000FEB01	Male Genitalia - 100%
171	LI:246422.1:2000FEB01	Hemic and Immune System - 100%
172	LG:449404.1:2000MAY19	Nervous System - 100%
173	LG:449413.1:2000MAY19	Nervous System - 100%
174	LG:450105.1:2000MAY19	Nervous System - 100%
175	LG:460809.1:2000MAY19	Exocrine Glands - 100%
176	LG:481781.1:2000MAY19	Nervous System - 100%
177	LG:1101153.1:2000MAY19	Nervous System - 100%
178	LI:257695.20:2000MAY01	Exocrine Glands - 28%, Endocrine System - 19%, Nervous System - 16%, Digestive System - 16%
179	LI:455771.1:2000MAY01	Nervous System - 100%
180	LI:274551.1:2000MAY01	Nervous System - 60%, Hemic and Immune System - 40%

181	LI:035973.1:2000MAY01	Embryonic Structures - 58%, Digestive System - 26%, Nervous System - 16%
182	LG:978427.5:2000FEB18	Nervous System - 100%
183	LG:247781.2:2000FEB18	Nervous System - 11%
184	LI:034583.1:2000FEB01	Nervous System - 35%, Endocrine System - 35% Cardiovascular System - 28%, Urinary Tract - 27%, Musculoskeletal System - 17%
185	LI:333307.2:2000FEB01	Respiratory System - 100%
186	LI:814710.2:2000FEB01	Endocrine System - 82%, Nervous System - 18%
187	LG:414732.1:2000MAY19	Connective Tissue - 55%, Nervous System - 15%, Embryonic Structures - 13%
188	LG:413910.6:2000MAY19	Endocrine System - 80%, Nervous System - 20%
189	LI:414732.2:2000MAY01	Urinary Tract - 15%, Male Genitalia - 12%
190	LI:900264.2:2000MAY01	Urinary Tract - 46%, Endocrine System - 17%, Germ Cells - 14%
191	LI:335593.1:2000MAY01	Stomatognathic System - 35%, Digestive System - 14%
192	LI:1189543.1:2000MAY01	Nervous System - 100%
193	LG:455450.1:2000FEB18	Nervous System - 100%
194	LG:1040978.1:2000FEB18	Liver - 80%, Hemic and Immune System - 13%
195	LG:446649.1:2000FEB18	Unclassified/Mixed - 17%, Sense Organs - 16%, Embryonic Structures - 10%
196	LG:132147.3:2000FEB18	Nervous System - 80%
197	LI:036034.1:2000FEB01	Unclassified/Mixed - 53%, Cardiovascular System - 21%, Nervous System - 16%
198	LG:162161.1:2000MAY19	Unclassified/Mixed - 40%, Respiratory System - 24%, Cardiovascular System - 16%
199	LG:407214.10:2000MAY19	Digestive System - 41%, Exocrine Glands - 24%, Female Genitalia - 18%
200	LG:204626.1:2000MAY19	Unclassified/Mixed - 31%, Nervous System - 25%, Urinary Tract - 11%
201	LI:007401.1:2000MAY01	Connective Tissue - 77%, Nervous System - 23%
202	LI:476342.1:2000MAY01	Hemic and Immune System - 27%, Musculoskeletal System - 19%, Endocrine System - 11%
203	LI:1072759.1:2000MAY01	Digestive System - 58%, Pancreas - 12%
204	LG:998857.1:2000FEB18	Male Genitalia - 85%, Respiratory System - 15%
205	LG:482261.1:2000FEB18	Skin - 20%, Germ Cells - 18%, Female Genitalia - 10%
206	LG:480328.1:2000FEB18	Germ Cells - 44%, Digestive System - 15%, Male Genitalia - 11%
207	LG:311197.1:2000MAY19	Endocrine System - 100%
208	LG:1054883.1:2000MAY19	Hemic and Immune System - 100%
209	LG:399395.1:2000MAY19	Germ Cells - 23%, Exocrine Glands - 14%, Connective Tissue - 13%
210	LG:380497.2:2000MAY19	Female Genitalia - 100%
211	LI:272913.22:2000MAY01	

TABLE 6

SEQ ID NO:	Frame	Length	Start	Stop	GI Number	Probability Score	Annotation
212	3	115	198	542	g399660	3.00E-51	aldehyde reductase [Rattus norvegicus]
212	3	115	198	542	g7677318	7.00E-51	aldehyde reductase [Mus musculus]
212	3	115	198	542	g6013149	2.00E-48	aldehyde reductase [Homo sapiens]
213	3	161	3	485	g2909424	2.00E-60	Glyoxalase I [Cicer arietinum]
213	3	161	3	485	g2113825	2.00E-58	Glyoxalase I [Brassica juncea]
213	3	161	3	485	g1177314	4.00E-57	glyoxalase-I [Lycopersicon esculentum]
214	2	332	2	997	g8671168	0	hypothetical protein [Homo sapiens]
214	2	332	2	997	g8886025	0	collapsin response mediator protein-5 [Homo sapiens]
214	2	332	2	997	g8671360	1.00E-179	Ulip-like protein [Rattus norvegicus]
215	3	274	12	833	g29600	2.00E-86	carbonic anhydrase I (AA 1-261) [Homo sapiens]
215	3	274	12	833	g179793	2.00E-86	carbonic anhydrase I (EC 4.2.1.1) [Homo sapiens]
215	3	274	12	833	g29587	4.00E-84	carbonic anhydrase II (AA 1-260) [Homo sapiens]
216	1	182	742	1287	g10438188	1.00E-102	unnamed protein product [Homo sapiens]
216	1	182	742	1287	g9949721	3.00E-49	probable acetyl-coa synthetase [Pseudomonas aeruginosa]
216	1	182	742	1287	g9655831	7.00E-46	prpE protein [Vibrio cholerae]
217	2	359	2	1078	g2104689	1.00E-111	alpha glucosidase II, alpha subunit [Mus musculus]
217	2	359	2	1078	g7672977	1.00E-111	glucosidase II alpha subunit [Homo sapiens]
							The ha1225 gene product is related to human alpha-glucosidase. [Homo sapiens]
217	2	359	2	1078	g577295	1.00E-110	sapiens]
218	2	110	161	490	g9653274	1.00E-26	ornithine decarboxylase-2 [Xenopus laevis]
218	2	110	161	490	g200124	5.00E-18	ornithine decarboxylase [Mus pahari]
218	2	110	161	490	g53518	1.00E-17	ornithine decarboxylase [Mus musculus]
219	3	549	36	1682	g10435462	0	unnamed protein product [Homo sapiens]
219	3	549	36	1682	g7023375	0	unnamed protein product [Homo sapiens]
219	3	549	36	1682	g10433608	1.00E-164	unnamed protein product [Homo sapiens]
220	1	264	1	792	g7023634	3.00E-92	unnamed protein product [Homo sapiens]
							similar to C. elegans R10H10.6 and S. cerevisiae YD8419.03c [Drosophila melanogaster]
220	1	264	1	792	g3213202	3.00E-49	CG2846 gene product [Drosophila melanogaster]
220	1	264	1	792	g7298960	3.00E-49	transglutaminase E3 [Homo sapiens]
221	3	701	33	2135	g307504	0	TGM3 (PROTEIN-GLUTAMINE GLUTAMYLTRANSFERASE E3
							PRECURSOR (EC 2.3.2.13) (TGASE E3) (TRANSGLUTAMINASE 3). [Homo sapiens]
221	3	701	33	2135	g4467804	0	transglutaminase E3 [Mus musculus]
221	3	701	33	2135	g309521	0	

222	2	150	2	451	g35505	7.00E-65	pyruvate kinase [Homo sapiens]
222	2	150	2	451	g189998	7.00E-65	M2-type pyruvate kinase [Homo sapiens]
222	2	150	2	451	g2623945	3.00E-64	pyruvate kinase; ATP-pyruvate 2-o-phosphotransferase [Oryctolagus cuniculus]
223	2	234	866	1567	g2576305	1.00E-128	anysulphatase [Homo sapiens]
223	2	234	866	1567	g791002	3.00E-82	ARSD [Homo sapiens]
223	2	234	866	1567	g791004	4.00E-75	ARSE [Homo sapiens]
224	2	86	2	259			
225	2	173	1049	1567	g4092820	8.00E-62	BC319430_7 [Homo sapiens]
225	2	173	1049	1567	g2792016	2.00E-54	olfactory receptor [Homo sapiens]
225	2	173	1049	1567	g4092819	2.00E-54	BC319430_5 [Homo sapiens]
226	2	68	86	289	g8272468	4.00E-15	envelope protein [Homo sapiens]
226	2	68	86	289	g4773880	4.00E-15	envelope protein precursor [Homo sapiens]
226	2	68	86	289	g4262296	4.00E-15	envelope protein [Homo sapiens]
227	1	70	79	288	g11231093	1.00E-11	hypothetical protein [Macaca fascicularis]
227	1	70	79	288	g10435559	3.00E-10	unnamed protein product [Homo sapiens]
227	1	70	79	288	g7020625	2.00E-09	unnamed protein product [Homo sapiens]
228	2	117	836	1186	g5726235	3.00E-18	unknown protein U5/2 [multiple sclerosis associated retrovirus element]
229	2	294	2	883	g404634	1.00E-59	serine/threonine kinase [Mus musculus]
229	2	294	2	883	g2738898	3.00E-59	protein kinase [Mus musculus]
229	2	294	2	883	g8101585	2.00E-54	testis specific serine kinase-3 [Mus musculus]
230	1	326	1	978	g2117166	1.00E-160	Ras like GTPase [Homo sapiens]
230	1	326	1	978	g466271	1.00E-140	Rar protein [Homo sapiens]
230	1	326	1	978	g3036779	1.00E-102	match: multiple proteins; RAR (RAS like GTPASE) like [Homo sapiens]
231	1	182	40	585	g5763838	1.00E-66	dJ593C16.1 (ras GTPase activating protein) [Homo sapiens]
231	1	182	40	585	g4417207	1.00E-66	synGAP-d [Rattus norvegicus]
231	1	182	40	585	g4105589	1.00E-66	nGAP [Homo sapiens]
232	1	358	58	1131	g1469876	1.00E-103	The KIAA0147 gene product is related to adenylyl cyclase. [Homo sapiens]
232	1	358	58	1131	g6850952	1.00E-86	vartul-2 protein [Drosophila melanogaster]
232	1	358	58	1131	g6782322	1.00E-86	Vartul-1 protein [Drosophila melanogaster]
233	1	194	370	951	g7008402	1.00E-107	kappa B-ras 1 [Homo sapiens]
233	1	194	370	951	g7239257	1.00E-103	kappaB-Ras1 [Mus musculus]
233	1	194	370	951	g7008404	8.00E-75	kappa B-ras 2 [Homo sapiens]
234	2	222	17	682	g9368448	1.00E-111	phospholipase C-beta-1a [Homo sapiens]
234	2	222	17	682	g9368450	1.00E-111	phospholipase C-beta-1b [Homo sapiens]
234	2	222	17	682	g206218	1.00E-110	phospholipase C-1 [Rattus sp.]

235	3	185	126	680	g3599940	1.00E-57	faciogenital dysplasia protein 2 [Mus musculus]
235	3	185	126	680	g10440426	8.00E-42	FLJ00048 protein [Homo sapiens]
235	3	185	126	680	g595425	4.00E-20	FGD1 [Homo sapiens]
236	2	192	707	1282			
237	3	61	204	386			
238	2	335	17	1021	g3005085	2.00E-92	hook1 protein [Homo sapiens]
238	2	335	17	1021	g5706448	2.00E-92	dJ782L23.1 (HOOK1) [Homo sapiens]
238	2	335	17	1021	g3005087	2.00E-70	hook2 protein [Homo sapiens]
239	1	346	1261	2298	g1109782	1.00E-105	protein-tyrosine phosphatase [Homo sapiens]
239	1	346	1261	2298	g1781037	1.00E-76	neuronal tyrosine threonine phosphatase 1 [Mus musculus]
239	1	346	1261	2298	g10241798	5.00E-11	hypothetical protein SCE41.24c [Streptomyces coelicolor]
240	3	298	147	1040	g4678722	1.00E-156	hypothetical protein [Homo sapiens]
							dJ272L16.1 (Rat Ca2+/Calmodulin dependent Protein Kinase LIKE protein)
240	3	298	147	1040	g4007153	1.00E-153	[Homo sapiens]
240	3	298	147	1040	g2077934	1.00E-152	Protein Kinase [Rattus norvegicus]
241	1	133	133	531	g10440426	1.00E-34	FLJ00048 protein [Homo sapiens]
241	1	133	133	531	g3599940	2.00E-16	faciogenital dysplasia protein 2 [Mus musculus]
242	2	354	821	1882	g11907572	1.00E-143	TSC22-related inducible leucine zipper 1b [Mus musculus]
242	2	354	821	1882	g1181619	1.00E-106	a variant of TSC-22 [Gallus gallus]
242	2	354	821	1882	g3327152	9.00E-16	KIAA0669 protein [Homo sapiens]
243	1	237	1	711	g6683492	1.00E-105	bromodomain PHD finger transcription factor [Homo sapiens]
							contains similarity to Pfam domain: PF00439 (Bromodomain), Score=125.5, E-value=1.5e-35, N=1; PF00628 (PHD-finger), Score=102.0, E-value=3.8e-27, N=2 [Caenorhabditis elegans]
243	1	237	1	711	g3876452	9.00E-53	N=2 [Caenorhabditis elegans]
							predicted using GeneFinder~contains similarity to Pfam domain: PF00439 (Bromodomain), Score=125.5, E-value=1.5e-35, N=1; PF00628 (PHD-finger), Score=102.0, E-value=3.8e-27, N=2 [Caenorhabditis elegans]
243	1	237	1	711	g3876449	9.00E-53	Score=102.0, E-value=3.8e-27, N=2 [Caenorhabditis elegans]
244	1	161	1	483	g6330736	1.00E-42	KIAA1234 protein [Homo sapiens]
244	1	161	1	483	g11244871	1.00E-40	dioxin receptor repressor [Homo sapiens]
244	1	161	1	483	g4164151	4.00E-35	AhR repressor [Mus musculus]
245	3	151	54	506	g10433955	9.00E-44	unnamed protein product [Homo sapiens]
245	3	151	54	506	g7295442	1.00E-16	CG17334 gene product [Drosophila melanogaster]
245	3	151	54	506	g2745892	1.00E-12	Y box transcription factor [Mus musculus]
							supported by Genscan and several ESTs: C83049 (NID:g3062006), AA823760 (NID:g2893628), AA215791 (NID:g1815572), A1095488 (NID:g3434464), and AA969095 (NID:g3144275) [Homo sapiens]
246	2	160	173	652	g3924670	4.00E-68	

246	2	160	173	652	g5640105	2.00E-59	homeobox protein LSX [Homo sapiens]
246	2	160	173	652	g6523391	6.00E-59	phf1 protein [Mus musculus]
247	3	160	108	587	g6939732	1.00E-52	transcription factor Elongin A2 [Homo sapiens]
247	3	160	108	587	g4581412	1.00E-29	dJ866K2.1 (elongin A; RNA polymerase; RNA polymerase II; RNA polymerase II elongation factor.) [Homo sapiens]
247	3	160	108	587	g992563	1.00E-29	elongin A [Homo sapiens]
248	1	171	25	537	g11907923	4.00E-29	enhancer of polycomb [Homo sapiens]
248	1	171	25	537	g3757890	3.00E-18	enhancer of polycomb [Drosophila melanogaster]
248	1	171	25	537	g7303589	3.00E-18	E(Pc) gene product [Drosophila melanogaster]
249	2	449	266	1612	g10443047	0	bA465L10.2 (novel C2H2 type zinc finger protein similar to chicken FZF-1) [Homo sapiens]
249	2	449	266	1612	g10438918	0	unnamed protein product [Homo sapiens]
249	2	449	266	1612	g984814	8.00E-98	zinc finger protein [Gallus gallus]
250	2	127	140	520	g10434195	2.00E-64	unnamed protein product [Homo sapiens]
250	2	127	140	520	g6467206	3.00E-36	gonadotropin inducible transcription repressor-4 [Homo sapiens]
250	2	127	140	520	g6330394	4.00E-34	KIAA1198 protein [Homo sapiens]
251	1	157	1	471	g340446	2.00E-17	zinc finger protein 7 (ZFP7) [Homo sapiens]
251	1	157	1	471	g4325310	2.00E-17	zinc-finger protein 7 [Homo sapiens]
251	1	157	1	471	g6007771	5.00E-17	KID2 [Mus musculus]
252	1	305	145	1059	g6002480	3.00E-49	BWSCR2 associated zinc-finger protein BAZ2 [Homo sapiens]
252	1	305	145	1059	g9963806	3.00E-47	zinc finger protein ZNF287 [Homo sapiens]
252	1	305	145	1059	g11527849	8.00E-43	zinc finger protein SKAT2 [Mus musculus]
253	2	717	305	2455	g10047335	0	KIAA1629 protein [Homo sapiens]
253	2	717	305	2455	g1504006	1.00E-96	similar to human ZFY protein. [Homo sapiens]
253	2	717	305	2455	g7243280	4.00E-66	KIAA1441 protein [Homo sapiens]
254	1	211	1	633	g10047183	3.00E-49	KIAA1559 protein [Homo sapiens]
254	1	211	1	633	g5080758	2.00E-45	BC331191_1 [Homo sapiens]
254	1	211	1	633	g498721	3.00E-44	zinc finger protein [Homo sapiens]
255	2	103	2	310	g498152	2.00E-20	ha0946 protein is Kruppel-related. [Homo sapiens]
255	2	103	2	310	g7576272	2.00E-20	bA393J16.1 (zinc finger protein 33a (KOX 31)) [Homo sapiens]
255	2	103	2	310	g10440081	2.00E-19	unnamed protein product [Homo sapiens]
256	3	84	135	386	g347906	2.00E-26	zinc finger protein [Homo sapiens]
256	3	84	135	386	g3342002	1.00E-25	hematopoietic cell derived zinc finger protein [Homo sapiens]
256	3	84	135	386	g8163824	5.00E-25	krueppel-like zinc finger protein HZF2 [Homo sapiens]
257	1	194	103	684	g10435738	4.00E-74	unnamed protein product [Homo sapiens]
257	1	194	103	684	g1017722	8.00E-73	repressor transcriptional factor [Homo sapiens]

257	1	194	103	684	g7959207	3.00E-71	KIAA1473 protein [Homo sapiens]
258	1	129	28	414	g2072955	6.00E-07	p40 [Homo sapiens]
258	1	129	28	414	g483915	8.00E-07	ORF1, encodes a 40 kDa product [Homo sapiens]
258	1	129	28	414	g339776	8.00E-07	ORF1 codes for a 40 kDa product [Homo sapiens]
259	3	93	75	353	g3329372	4.00E-36	DNA-binding protein [Homo sapiens]
259	3	93	75	353	g7959207	1.00E-33	KIAA1473 protein [Homo sapiens]
259	3	93	75	353	g184452	3.00E-33	Kruppel-related DNA-binding protein [Homo sapiens]
260	3	193	369	947	g8099348	1.00E-38	zinc finger protein [Homo sapiens]
260	3	193	369	947	g5730196	2.00E-38	Kruppel-type zinc finger [Homo sapiens]
260	3	193	369	947	g8050899	4.00E-38	ZNF180 [Homo sapiens]
261	3	111	3	335	g7023216	1.00E-14	unnamed protein product [Homo sapiens]
261	3	111	3	335	g3406676	6.00E-14	zinc finger protein 54 [Mus musculus]
261	3	111	3	335	g9802037	3.00E-13	zinc finger protein SBZF3 [Homo sapiens]
262	3	137	75	485	g186774	1.00E-26	zinc finger protein [Homo sapiens]
262	3	137	75	485	g2384653	6.00E-26	Kruppel family zinc finger protein [Homo sapiens]
262	3	137	75	485	g8163824	6.00E-26	kruppel-like zinc finger protein HZF2 [Homo sapiens]
263	3	68	51	254	g7239109	2.00E-15	HSPC059 [Homo sapiens]
263	3	68	51	254	g347906	4.00E-15	zinc finger protein [Homo sapiens]
263	3	68	51	254	g7023216	2.00E-14	unnamed protein product [Homo sapiens]
264	3	101	90	392	g3329372	8.00E-35	DNA-binding protein [Homo sapiens]
264	3	101	90	392	g4559318	7.00E-32	BC273239_1 [Homo sapiens]
264	3	101	90	392	g184452	9.00E-32	Kruppel-related DNA-binding protein [Homo sapiens]
265	1	96	184	471	g4589588	5.00E-22	KIAA0972 protein [Homo sapiens]
265	1	96	184	471	g4514561	6.00E-22	KRAB-containing zinc-finger protein KRAZ2 [Mus musculus]
265	1	96	184	471	g7576272	2.00E-21	bA393J16.1 (zinc finger protein 33a (KOX 31)) [Homo sapiens]
266	2	251	2	754	g55471	1.00E-134	Zfp-29 [Mus musculus]
266	2	251	2	754	g1020145	3.00E-73	DNA binding protein [Homo sapiens]
266	2	251	2	754	g6002478	3.00E-72	BWSCR2 associated zinc-finger protein BAZ1 [Homo sapiens]
267	3	522	36	1601	g10443047	0	bA465L10.2 (novel C2H2 type zinc finger protein similar to chicken FZF-1) [Homo sapiens]
267	3	522	36	1601	g10438918	0	unnamed protein product [Homo sapiens]
267	3	522	36	1601	g984814	2.00E-97	zinc finger protein [Gallus gallus]
268	2	267	2	802	g9886891	4.00E-45	zinc finger protein 276 C2H2 type [Mus musculus]
268	2	267	2	802	g11611571	3.00E-43	hypothetical protein [Macaca fascicularis]
268	2	267	2	802	g453376	4.00E-43	zinc finger protein PZF [Mus musculus]
269	2	286	2	859	g2754696	9.00E-08	high molecular mass nuclear antigen [Gallus gallus]

269	2	286	2	859	g2078483	9.00E-06	antifreeze glycopeptide AFGP polyprotein precursor [Boreogadus saida] PR-domain zinc finger protein 6 isoform A; PR-domain family protein 3 isoform A; PRDM6A; PFM3A [Homo sapiens]
270	3	194	270	851	g8575782	1.00E-112	unnamed protein product [Homo sapiens]
270	3	194	270	851	g10437767	1.00E-26	CG15436 gene product [Drosophila melanogaster]
270	3	194	270	851	g7295698	9.00E-26	zinc finger protein ZNF180 [Homo sapiens]
271	3	263	3	791	g6409345	1.00E-107	ZNF180 [Homo sapiens]
271	3	263	3	791	g8050899	1.00E-107	pMLZ-4 [Mus musculus]
271	3	263	3	791	g200407	1.00E-101	Eos protein [Mus musculus]
272	2	142	290	715	g4062983	5.00E-65	eos [Raja eglanteria]
272	2	142	290	715	g9408382	4.00E-46	zinc finger transcription factor Eos [Homo sapiens]
272	2	142	290	715	g11612390	3.00E-42	KRAB zinc finger protein; Method: conceptual translation supplied by author [Homo sapiens]
273	2	164	2	493	g1049301	3.00E-25	KIAA1588 protein [Homo sapiens]
273	2	164	2	493	g10047251	9.00E-25	KRAB zinc finger protein [Mus musculus]
273	2	164	2	493	g8809810	1.00E-19	zinc finger protein [Cavia porcellus]
274	2	107	509	829	g1237278	2.00E-36	unnamed protein product [Homo sapiens]
274	2	107	509	829	g7023417	4.00E-36	HPF1 protein [Homo sapiens]
274	2	107	509	829	g11917507	5.00E-36	ba508N22.2 (zinc finger protein 37a (KOX 21)) [Homo sapiens]
275	3	105	336	650	g9801232	2.00E-51	ZNF37A [Homo sapiens]
275	3	105	336	650	g829151	2.00E-51	Kruppel-type zinc finger [Homo sapiens]
275	3	105	336	650	g5730196	4.00E-36	Zinc finger protein 222 [Homo sapiens]
276	1	149	1	447	g7656698	3.00E-91	zinc finger protein ZNF222 [Homo sapiens]
276	1	149	1	447	g6118381	3.00E-91	zinc finger protein ZNF223 [Homo sapiens]
276	1	149	1	447	g6118383	1.00E-81	DNA-binding protein [Homo sapiens]
277	3	101	90	392	g3329372	1.00E-30	BC273239_1 [Homo sapiens]
277	3	101	90	392	g4559318	3.00E-29	Kruppel-related DNA-binding protein [Homo sapiens]
277	3	101	90	392	g1124876	5.00E-29	ba245E14.1 (novel zinc finger protein similar to ZFP47) [Homo sapiens]
278	3	137	6	416	g11062533	2.00E-46	zinc finger protein ZFP113 [Mus musculus]
278	3	137	6	416	g5640017	2.00E-46	zinc finger protein [Homo sapiens]
278	3	137	6	416	g186774	5.00E-46	ZNF37A [Homo sapiens]
279	3	97	165	455	g829151	2.00E-27	ba508N22.2 (zinc finger protein 37a (KOX 21)) [Homo sapiens]
279	3	97	165	455	g9801232	2.00E-27	dJ733D15.1 (Zinc-finger protein) [Homo sapiens]
279	3	97	165	455	g3702137	9.00E-20	ba508N22.2 (zinc finger protein 37a (KOX 21)) [Homo sapiens]
280	2	97	182	472	g9801232	4.00E-29	ZNF37A [Homo sapiens]
280	2	97	182	472	g829151	4.00E-29	pMLZ-4 [Mus musculus]
280	2	97	182	472	g200407	4.00E-21	

281	1	179	31	567	g10442700	3.00E-61	zinc-finger protein ZBRK1 [Homo sapiens]
281	1	179	31	567	g10435411	3.00E-61	unnamed protein product [Homo sapiens]
281	1	179	31	567	g10954044	3.00E-61	KRAB zinc finger protein ZFQR [Homo sapiens]
282	3	87	369	629	g8099348	2.00E-14	zinc finger protein [Homo sapiens]
282	3	87	369	629	g498725	2.00E-14	zinc finger protein [Homo sapiens]
282	3	87	369	629	g495568	2.00E-13	zinc finger protein [Homo sapiens]
283	2	172	2	517	g6007771	4.00E-97	KID2 [Mus musculus]
283	2	172	2	517	g2970038	2.00E-93	HKL1 [Homo sapiens]
283	2	172	2	517	g205067	2.00E-93	zinc finger protein [Rattus norvegicus]
284	1	151	1	453	g1806134	5.00E-57	zinc finger protein [Mus musculus]
284	1	151	1	453	g538413	5.00E-57	zinc finger protein [Mus musculus]
284	1	151	1	453	g186774	3.00E-55	zinc finger protein [Homo sapiens]
285	2	89	83	349	g7023216	2.00E-18	unnamed protein product [Homo sapiens]
285	2	89	83	349	g9802037	4.00E-16	zinc finger protein SBZF3 [Homo sapiens]
285	2	89	83	349	g7239109	7.00E-15	HSPC059 [Homo sapiens]
286	2	146	62	499	g2739353	7.00E-56	ZNF91L [Homo sapiens]
286	2	146	62	499	g7959207	5.00E-50	KIAA1473 protein [Homo sapiens]
286	2	146	62	499	g3342002	7.00E-50	hematopoietic cell derived zinc finger protein [Homo sapiens]
287	1	78	1	234	g487785	4.00E-16	zinc finger protein ZNF136 [Homo sapiens]
287	1	78	1	234	g5262560	7.00E-15	hypothetical protein [Homo sapiens]
287	1	78	1	234	g10434856	9.00E-15	unnamed protein product [Homo sapiens]
288	3	126	78	455	g9963804	4.00E-47	zinc finger protein ZNF286 [Homo sapiens]
288	3	126	78	455	g5640017	2.00E-46	zinc finger protein ZFP113 [Mus musculus]
288	3	126	78	455	g7020166	4.00E-46	unnamed protein product [Homo sapiens]
289	1	96	151	438	g4589588	5.00E-22	KIAA0972 protein [Homo sapiens]
289	1	96	151	438	g4514561	6.00E-22	KRAB-containing zinc-finger protein KRAZ2 [Mus musculus]
289	1	96	151	438	g7576272	2.00E-21	bA393J16.1 (zinc finger protein 33a (KOX 31)) [Homo sapiens]
290	1	149	118	564	g7959207	1.00E-26	KIAA1473 protein [Homo sapiens]
290	1	149	118	564	g498736	3.00E-26	zinc finger protein [Homo sapiens]
290	1	149	118	564	g4454678	4.00E-23	zinc finger protein 4 [Homo sapiens]
291	2	134	152	553	g498152	1.00E-06	ha0946 protein is Kruppel-related. [Homo sapiens]
291	2	134	152	553	g10440081	1.00E-06	unnamed protein product [Homo sapiens]
291	2	134	152	553	g7576272	1.00E-06	bA393J16.1 (zinc finger protein 33a (KOX 31)) [Homo sapiens]
292	2	212	2	637	g7656698	1.00E-133	Zinc finger protein 222 [Homo sapiens]
292	2	212	2	637	g6118381	1.00E-133	zinc finger protein ZNF222 [Homo sapiens]
292	2	212	2	637	g6118383	1.00E-122	zinc finger protein ZNF223 [Homo sapiens]

293	2	108	2	325	g4567179	2.00E-33	BC37295_1 [Homo sapiens]
293	2	108	2	325	g10434142	9.00E-31	unnamed protein product [Homo sapiens]
293	2	108	2	325	g5817149	9.00E-31	hypothetical protein [Homo sapiens]
294	1	83	97	345	g930123	9.00E-24	zinc finger protein (583 AA) [Homo sapiens]
294	1	83	97	345	g487785	8.00E-23	zinc finger protein ZNF136 [Homo sapiens]
294	1	83	97	345	g5262560	1.00E-22	hypothetical protein [Homo sapiens]
295	1	180	1	540	g498719	2.00E-83	zinc finger protein [Homo sapiens]
295	1	180	1	540	g3953593	3.00E-69	Zinc finger protein s11-6 [Mus musculus]
295	1	180	1	540	g6467206	4.00E-68	gonadotropin inducible transcription repressor-4 [Homo sapiens]
296	3	97	57	347	g9801232	3.00E-28	ba508N22.2 (zinc finger protein 37a (KOX 21)) [Homo sapiens]
296	3	97	57	347	g829151	3.00E-28	ZNF37A [Homo sapiens]
296	3	97	57	347	g881564	4.00E-20	ZNF157 [Homo sapiens]
297	1	217	421	1071	g6331377	1.00E-131	KIAA1285 protein [Homo sapiens]
297	1	217	421	1071	g1020145	6.00E-53	DNA binding protein [Homo sapiens]
297	1	217	421	1071	g2224593	1.00E-52	KIAA0326 [Homo sapiens]
298	3	137	15	425	g4456989	4.00E-20	protease [Homo sapiens]
298	3	137	15	425	g9558703	4.00E-20	protease [Homo sapiens]
298	3	137	15	425	g1780976	5.00E-20	protease [Human endogenous retrovirus K]
299	2	169	59	565	g10434856	2.00E-40	unnamed protein product [Homo sapiens]
299	2	169	59	565	g5262560	2.00E-40	hypothetical protein [Homo sapiens]
299	2	169	59	565	g930123	1.00E-31	zinc finger protein (583 AA) [Homo sapiens]
300	3	135	3	407	g10434856	3.00E-35	unnamed protein product [Homo sapiens]
300	3	135	3	407	g5262560	3.00E-35	hypothetical protein [Homo sapiens]
300	3	135	3	407	g10434195	2.00E-27	unnamed protein product [Homo sapiens]
301	1	170	22	531	g10047297	2.00E-23	KIAA1611 protein [Homo sapiens]
301	1	170	22	531	g7023216	2.00E-22	unnamed protein product [Homo sapiens]
301	1	170	22	531	g347906	5.00E-16	zinc finger protein [Homo sapiens]
302	3	181	3	545	g5931821	8.00E-79	dJ228H13.3 (zinc finger protein) [Homo sapiens]
302	3	181	3	545	g6807587	8.00E-79	hypothetical protein [Homo sapiens]
302	3	181	3	545	g488555	2.00E-63	zinc finger protein ZNF135 [Homo sapiens]
303	1	263	1	789	g506502	1.00E-141	NK10 [Mus musculus]
303	1	263	1	789	g488555	1.00E-92	zinc finger protein ZNF135 [Homo sapiens]
303	1	263	1	789	g8453103	7.00E-88	zinc finger protein [Homo sapiens]
304	3	340	18	1037	g7023216	1.00E-142	unnamed protein product [Homo sapiens]
304	3	340	18	1037	g7023703	2.00E-89	unnamed protein product [Homo sapiens]
304	3	340	18	1037	g10436789	7.00E-54	unnamed protein product [Homo sapiens]

305	1	89	103	369	g7023216	2.00E-18	unnamed protein product [Homo sapiens]
305	1	89	103	369	g9802037	4.00E-16	zinc finger protein SBZF3 [Homo sapiens]
305	1	89	103	369	g7239109	7.00E-15	HSPC059 [Homo sapiens]
306	1	80	1	240	g7959865	9.00E-20	PRO2032 [Homo sapiens]
306	1	80	1	240	g8099520	6.00E-11	muscleblind [Mus musculus]
306	1	80	1	240	g8515711	2.00E-10	EXP35 [Homo sapiens]
307	2	386	176	1333	g3869259	0	ZNF202 beta [Homo sapiens]
307	2	386	176	1333	g7328045	0	hypothetical protein [Homo sapiens]
307	2	386	176	1333	g5360097	1.00E-123	putative kruppel-related zinc finger protein NY-REN-23 antigen [Homo sapiens]
308	2	368	71	1174	g3882241	0	KIAA0760 protein [Homo sapiens]
308	2	368	71	1174	g6760445	0	Smad- and Olf-interacting zinc finger protein [Homo sapiens]
308	2	368	71	1174	g2149792	0	Roaz [Rattus norvegicus]
309	2	175	191	715	g487787	8.00E-15	zinc finger protein ZNF140 [Homo sapiens]
309	2	175	191	715	g10047183	9.00E-31	KIAA1559 protein [Homo sapiens]
309	2	175	191	715	g4567179	2.00E-29	BC37295_1 [Homo sapiens]
310	2	78	521	754			
311	1	61	394	576			
312	1	73	172	390	g2587027	4.00E-13	HERV-E envelope glycoprotein [Homo sapiens]
312	1	73	172	390	g2587024	4.00E-13	HERV-E envelope glycoprotein [Homo sapiens]
312	1	73	172	390	g1049232	2.00E-10	HERV-E envelope protein [Human endogenous retrovirus]
313	1	184	304	855	g8132311	2.00E-74	inwardly-rectifying potassium channel Kir5.1 [Homo sapiens]
313	1	184	304	855	g8132295	2.00E-74	inwardly-rectifying potassium channel Kir5.1 [Homo sapiens]
313	1	184	304	855	g8132293	2.00E-74	inwardly-rectifying potassium channel Kir5.1 [Homo sapiens]
314	2	219	164	820	g7105926	2.00E-22	calcium channel alpha2-delta3 subunit [Homo sapiens]
314	2	219	164	820	g4186073	2.00E-22	calcium channel alpha2-delta-C subunit [Mus musculus]
314	2	219	164	820	g9929977	2.00E-22	hypothetical protein [Macaca fascicularis]
315	1	1603	1	4809	g184039	0	sodium channel alpha subunit [Homo sapiens]
315	1	1603	1	4809	g6782382	0	voltage-gated sodium channel [Mus musculus]
315	1	1603	1	4809	g206858	0	sodium channel alpha-subunit [Rattus norvegicus]
316	3	200	240	839	g913242	5.00E-71	gamma-aminobutyric acid transporter type 3, GABA transporter type 3, GAT-3 [human, fetal brain, Peptide, 632 aa] [Homo sapiens]
316	3	200	240	839	g204220	2.00E-69	beta-alanine-sensitive neuronal GABA transporter [Rattus norvegicus]
316	3	200	240	839	g202535	2.00E-69	GABA transporter [Rattus norvegicus]
317	3	329	3	989	g6996442	4.00E-61	CTL1 protein [Homo sapiens]
317	3	329	3	989	g6996589	1.00E-59	CTL1 protein [Rattus norvegicus]

317	3	329	3	989	g6996587	2.00E-51	CTL1 protein [Torpedo marmorata] ESTs AU058081(E30812),AU058365(E50679), AU030138(E50679) correspond to a region of the predicted gene.; Similar to Spinacia oleracea mRNA for proteasome 37kD subunit.(X96974) [Oryza sativa] ESTs AU058081(E3082),AU075427(E30384) correspond to a region of the predicted gene.~Similar to Spinacia oleracea proteasome 27 kD subunit (P52427) [Oryza sativa] ESTs AU058081(E3082),AU075427(E30384) correspond to a region of the predicted gene.~Similar to Spinacia oleracea proteasome 27 kD subunit (P52427) [Oryza sativa]
318	3	256	3	770	g5091520	1.00E-134	housekeeping protein [Rattus norvegicus] cyclophilin A [Mus musculus] cyclophilin (AA 1 - 164) [Mus musculus] unnamed protein product [Homo sapiens] mDj10 [Mus musculus] unnamed protein product [Homo sapiens] HERV-E envelope protein [Human endogenous retrovirus] HERV-E envelope glycoprotein [Homo sapiens] HERV-E envelope glycoprotein [Homo sapiens] testis specific DNAj-homolog [Mus musculus] DnaJ homolog [Homo sapiens] DNAj homolog [Homo sapiens]
318	3	256	3	770	g8096329	1.00E-134	25 kDa trypsin inhibitor [Homo sapiens] dJ881L22.3 (novel protein similar to a trypsin inhibitor) [Homo sapiens] late gestation lung protein 1 [Rattus norvegicus] putative chaperonin [Arabidopsis thaliana] TCP-1 chaperonin-like protein [Arabidopsis thaliana] chaperonin containing TCP-1 zeta-1 subunit [Mus musculus] KIAA0723 protein [Homo sapiens] similar to Homo sapiens mRNA for KIAA0723 protein with GenBank Accession Number AB018266.1 [] matrin 3 [Homo sapiens]
318	3	256	3	770	g8096319	1.00E-134	proliferation-associated SNF2-like protein [Homo sapiens] lymphocyte specific helicase [Mus musculus]
319	2	76	2	229	g951425	2.00E-07	
319	2	76	2	229	g5759144	2.00E-07	
319	2	76	2	229	g50621	2.00E-07	
320	3	276	354	1181	g7019854	1.00E-84	
320	3	276	354	1181	g6567172	7.00E-84	
320	3	276	354	1181	g10436329	5.00E-81	
321	1	115	328	672	g1049232	3.00E-24	
321	1	115	328	672	g2587024	2.00E-23	
321	1	115	328	672	g2587027	2.00E-23	
322	3	227	3	683	g2286123	6.00E-33	
322	3	227	3	683	g6681592	1.00E-32	
322	3	227	3	683	g6648623	1.00E-32	
323	3	100	153	452			
324	3	142	840	1265	g2943716	5.00E-81	
324	3	142	840	1265	g9885193	5.00E-54	
324	3	142	840	1265	g4324682	2.00E-52	
325	3	263	3	791	g6957716	1.00E-135	
325	3	263	3	791	g9755653	1.00E-132	
325	3	263	3	791	g5295933	2.00E-93	
326	2	357	23	1093	g3882167	1.00E-171	
326	2	357	23	1093	g9956070	1.00E-171	
326	2	357	23	1093	g6563246	1.00E-170	
327	2	100	656	955			
328	2	303	2	910	g8980660	1.00E-158	
328	2	303	2	910	g805296	1.00E-149	

328	2	303	2	910	g9956001	8.00E-86	similar to Mus musculus lymphocyte specific helicase mRNA with GenBank Accession Number U25691.1 [Homo sapiens]
329	2	72	167	382			
330	2	76	80	307			
331	2	74	446	667	g2104910	1.00E-29	ORF derived from D1 leader region and integrase coding region [Homo sapiens]
331	2	74	446	667	g2104914	5.00E-21	ORF derived from protease and integrase coding regions [Homo sapiens]
331	2	74	446	667	g4959374	5.00E-21	pol protein [Homo sapiens]
332	3	67	57	257			
333	2	192	302	877	g8980660	8.00E-92	proliferation-associated SNF2-like protein [Homo sapiens]
333	2	192	302	877	g9956001	8.00E-92	similar to Mus musculus lymphocyte specific helicase mRNA with GenBank Accession Number U25691.1 [Homo sapiens]
333	2	192	302	877	g7022306	1.00E-89	unnamed protein product [Homo sapiens]
334	2	74	446	667	g2104910	1.00E-30	ORF derived from D1 leader region and integrase coding region [Homo sapiens]
334	2	74	446	667	g2104914	5.00E-21	ORF derived from protease and integrase coding regions [Homo sapiens]
334	2	74	446	667	g4959374	5.00E-21	pol protein [Homo sapiens]
335	2	72	167	382			orf; encodes putative chimeric protein with SET domain in N-terminus with similarity to several other human, Drosophila, nematode and yeast proteins [Homo sapiens]
336	2	55	557	721	g2231380	8.00E-12	unknown [Homo sapiens]
336	2	55	557	721	g3005702	8.00E-12	mariner transposase [Homo sapiens]
336	2	55	557	721	g1263081	1.00E-11	
337	3	107	1614	1934			
338	3	147	63	503	g10047265	7.00E-81	KIAA1595 protein [Homo sapiens]
338	3	147	63	503	g10176757	3.00E-26	ATP-dependent RNA helicase-like protein [Arabidopsis thaliana]
338	3	147	63	503	g3776011	3.00E-26	RNA helicase [Arabidopsis thaliana]
339	1	257	199	969	g10434055	1.00E-128	unnamed protein product [Homo sapiens]
339	1	257	199	969	g7243213	1.00E-126	KIAA1416 protein [Homo sapiens]
339	1	257	199	969	g11345539	1.00E-120	dj620E11.1 (novel Helicase C-terminal domain and SNF2 N-terminal domains containing protein, similar to KIAA0308) [Homo sapiens]
340	3	63	3	191			
341	1	112	1639	1974			
342	3	427	2097	3377	g2599502	0	protocadherin 68 [Homo sapiens]
342	3	427	2097	3377	g7243181	4.00E-49	KIAA1400 protein [Homo sapiens]
342	3	427	2097	3377	g4099551	5.00E-48	OL-protocadherin [Mus musculus]

343	2	144	635	1066	g10436424	1.00E-10	unnamed protein product [Homo sapiens]
344	2	97	557	847			
345	3	75	675	899	g2587027	4.00E-13	HERV-E envelope glycoprotein [Homo sapiens]
345	3	75	675	899	g2587024	4.00E-13	HERV-E envelope glycoprotein [Homo sapiens]
345	3	75	675	899	g1049232	2.00E-10	HERV-E envelope protein [Human endogenous retrovirus]
346	3	135	399	803	g9368839	2.00E-71	hypothetical protein [Homo sapiens]
346	3	135	399	803	g2739452	6.00E-58	ribosomal protein L23A [Homo sapiens]
346	3	135	399	803	g1399086	6.00E-58	ribosomal protein L23a [Homo sapiens]
347	2	55	179	343			
348	2	129	425	811	g11493463	2.00E-22	PRO2852 [Homo sapiens]
348	2	129	425	811	g9280152	5.00E-22	unnamed portein product [Macaca fascicularis]
348	2	129	425	811	g10437485	5.00E-21	unnamed protein product [Homo sapiens]
349	2	291	122	994	g673417	1.00E-152	class II antigen [Homo sapiens]
349	2	291	122	994	g703089	1.00E-152	MHC class II DP3-alpha [Homo sapiens]
349	2	291	122	994	g758100	1.00E-137	SB classII histocompatibility antigen alpha- chain [Homo sapiens]
							cytochrome P450 2B-Bx=phenobarbital-inducible [rabbits, kidney, Peptide, 491
350	1	517	1	1551	g402843	1.00E-144	aa] [Oryctolagus cuniculus]
350	1	517	1	1551	g404777	1.00E-144	cytochrome P-450 2B-Bx [Oryctolagus cuniculus]
350	1	517	1	1551	g164959	1.00E-142	cytochrome P-450 [Oryctolagus cuniculus]
351	1	232	1300	1995			
							dJ857M17.2 (novel protein similar to cytochrome c oxidase subunit IV
352	1	220	67	726	g11863734	2.00E-80	(COX4)) [Homo sapiens]
352	1	220	67	726	g8809758	9.00E-42	cytochrome c oxidase subunit IV isoform 2 precursor [Thunnus obesus]
352	1	220	67	726	g2809498	3.00E-41	cytochrome c oxidase subunit IV [Gorilla gorilla]
353	1	95	1	285			
354	2	331	2	994	g11229985	1.00E-176	unnamed protein product [Homo sapiens]
354	2	331	2	994	g11229992	6.00E-57	unnamed protein product [Mus musculus]
354	2	331	2	994	g30095	6.00E-49	collagen subunit (alpha-1 (X)) 3 [Homo sapiens]
355	3	93	54	332	g1177164	4.00E-12	polydom protein [Mus musculus]
355	3	93	54	332	g391669	4.00E-07	hikaru genki type4 product precursor [Drosophila melanogaster]
355	3	93	54	332	g391667	4.00E-07	hikaru genki type3 product precursor [Drosophila melanogaster]
356	1	112	1	336			
357	3	73	192	410			
							dJ708F5.1 (PUTATIVE novel Collagen alpha 1 LIKE protein) [Homo sapiens]
358	1	239	181	897	g4582324	1.00E-129	cartilage matrix protein [Homo sapiens]
358	1	239	181	897	g1732121	4.00E-36	

358	1	239	181	897	g180654	2.00E-35	cartilage matrix protein [Homo sapiens]
359	1	528	4	1587	g1903218	0	type II intermediate filament of hair keratin [Homo sapiens]
359	1	528	4	1587	g7161771	0	keratin [Homo sapiens]
359	1	528	4	1587	g4103156	0	hair keratin basic 5; keratin Hb5 [Mus musculus]
360	2	157	161	631	g11034725	2.00E-64	hNBL4 [Homo sapiens]
360	2	157	161	631	g466548	3.00E-63	NBL4 [Mus musculus]
360	2	157	161	631	g2822458	5.00E-54	band 4.1-like protein 4 [Danio rerio]
361	3	65	54	248	g3724141	6.00E-08	myosin I [Rattus norvegicus]
361	3	65	54	248	g3882175	6.00E-08	KIAA0727 protein [Homo sapiens]
							dJ111C20.1 (similar to Chlamydomonas radial spoke protein 3) [Homo sapiens]
362	3	517	3	1553	g6855339	1.00E-120	spoke protein [Chlamydomonas reinhardtii]
362	3	517	3	1553	g18218	1.00E-75	CG10099 gene product [Drosophila melanogaster]
362	3	517	3	1553	g7295323	9.00E-47	
363	2	60	314	493			PF20 [Chlamydomonas reinhardtii]
364	1	239	127	843	g1813638	9.00E-53	pf20 homolog [Trypanosoma brucei]
364	1	239	127	843	g3983133	2.00E-47	beta transducin-like protein [Podospora anserina]
364	1	239	127	843	g607003	1.00E-37	
365	1	160	1	480			kinesin-like protein GAKIN [Homo sapiens]
366	3	757	3	2273	g8896164	0	KIF13A [Mus musculus]
366	3	757	3	2273	g10697238	0	kinesin-like protein RBKIN2 [Homo sapiens]
366	3	757	3	2273	g11761613	0	hypothetical protein [Macaca fascicularis]
367	3	162	3	488	g11231085	1.00E-56	ankyrin 1 [Bos taurus]
367	3	162	3	488	g7385113	2.00E-18	alt. ankyrin (variant 2.2) [Homo sapiens]
367	3	162	3	488	g747710	2.00E-18	dystrophin-related protein 2 [Homo sapiens]
368	2	635	308	2212	g1353782	0	dystrophin-related protein 2 A-form splice variant [Rattus norvegicus]
368	2	635	308	2212	g11066165	0	dystrophin-related protein 2 B-form splice variant [Rattus norvegicus]
368	2	635	308	2212	g11066167	0	pemphigus vulgaris antigen [Homo sapiens]
369	3	433	999	2297	g190752	0	desmoglein 3 [Mus musculus]
369	3	433	999	2297	g2290200	1.00E-176	desmoglein 2 [Homo sapiens]
369	3	433	999	2297	g416178	2.00E-58	64 Kd autoantigen [Homo sapiens]
370	3	531	3	1595	g28989	7.00E-71	tropomodulin 2 [Homo sapiens]
370	3	531	3	1595	g6934240	8.00E-61	neural tropomodulin N-Tmod [Mus musculus]
370	3	531	3	1595	g7288857	3.00E-60	The KIAA0143 gene product is related to a putative C.elegans gene encoded on cosmid C32D5. [Homo sapiens]
371	2	257	383	1153	g1469868	1.00E-124	KIAA0953 protein [Homo sapiens]
371	2	257	383	1153	g4589550	4.00E-82	

371	2	257	383	1153	g7304005	1.00E-55	cmp44E gene product [alt 1] [Drosophila melanogaster]
372	1	242	139	864	g387514	1.00E-123	DM-20 protein [Mus musculus]
372	1	242	139	864	g190088	1.00E-123	DM-20 [Homo sapiens]
372	1	242	139	864	g200409	1.00E-122	proteolipid protein variant Dm-20 [Mus musculus]
373	2	60	380	559			
374	1	157	22	492	g7268562	1.00E-59	ribosomal protein L32-like protein [Arabidopsis thaliana]
374	1	157	22	492	g5816996	1.00E-59	ribosomal protein L32-like protein [Arabidopsis thaliana]
374	1	157	22	492	g10177580	7.00E-59	ribosomal protein L32 [Arabidopsis thaliana]
375	3	158	3	476	g643074	4.00E-76	putative 40S ribosomal protein s12 [Fragaria x ananassa]
375	3	158	3	476	g6716785	1.00E-75	40s ribosomal protein S23 [Euphorbia esula]
375	3	158	3	476	g7413571	6.00E-75	putative protein [Arabidopsis thaliana]
376	2	238	14	727	g10799832	1.00E-93	ribosomal protein L11-like [Nicotiana tabacum]
376	2	238	14	727	g7630065	4.00E-93	ribosomal protein L11-like [Arabidopsis thaliana]
376	2	238	14	727	g11908058	4.00E-93	ribosomal protein L11, cytosolic [Arabidopsis thaliana]
377	3	102	3	308	g57131	7.00E-41	ribosomal protein S26 [Rattus norvegicus]
377	3	102	3	308	g296452	7.00E-41	ribosomal protein S26 [Homo sapiens]
377	3	102	3	308	g3335024	7.00E-41	ribosomal protein S26 [Homo sapiens]
378	1	102	316	621	g6969165	6.00E-53	dj475N16.3 (novel protein similar to RPL7A (60S ribosomal protein L7A))
378	1	102	316	621	g6687301	2.00E-21	[Homo sapiens]
378	1	102	316	621	g200785	1.00E-20	60S ribosomal protein L7 [Cyanophora paradoxa]
379	3	177	3	533	g206736	1.00E-82	ribosomal protein L7 [Mus musculus]
379	3	177	3	533	g200785	2.00E-80	ribosomal protein L7 [Rattus norvegicus]
379	3	177	3	533	g554269	2.00E-80	ribosomal protein L7 [Mus musculus]
380	2	86	257	514	g550025	2.00E-31	ribosomal protein L7 [Mus musculus]
380	2	86	257	514	g57127	3.00E-30	ribosomal protein S10 [Homo sapiens]
380	2	86	257	514	g9581772	3.00E-29	ribosomal protein S10 (AA 1-165) [Rattus norvegicus]
381	1	97	286	576	g36140	2.00E-31	bA371L19.2 (similar to ribosomal protein S10) [Homo sapiens]
381	1	97	286	576	g307388	2.00E-31	ribosomal protein L7 [Homo sapiens]
381	1	97	286	576	g1335288	2.00E-31	ribosomal protein L7 [Homo sapiens]
382	1	82	70	315	g409074	2.00E-19	ribosomal protein L7 [Homo sapiens]
382	1	82	70	315	g409072	2.00E-19	HBp15/L22 [Sus scrofa]
382	1	82	70	315	g409070	2.00E-19	HBp15/L22 [Mus musculus]
383	1	180	46	585	g4886269	2.00E-75	HBp15/L22 [Homo sapiens]
383	1	180	46	585	g6006890	6.00E-75	putative ribosomal protein S14 [Arabidopsis thaliana]
383	1	180	46	585	g4678226	3.00E-74	putative 40S ribosomal protein s14; 67401-66292 [Arabidopsis thaliana]
							40S ribosomal protein S14 [Arabidopsis thaliana]

384	3	118	21	374	g643074	2.00E-49	putative 40S ribosomal protein s12 [Fragaria x ananassa]
384	3	118	21	374	g6716785	6.00E-49	40s ribosomal protein S23 [Euphorbia esula]
384	3	118	21	374	g7413571	3.00E-48	putative protein [Arabidopsis thaliana]
385	2	164	2	493	g643074	4.00E-76	putative 40S ribosomal protein s12 [Fragaria x ananassa]
385	2	164	2	493	g6716785	1.00E-75	40s ribosomal protein S23 [Euphorbia esula]
385	2	164	2	493	g7413571	6.00E-75	putative protein [Arabidopsis thaliana]
386	3	101	3	305	g36130	1.00E-22	ribosomal protein L31 (AA 1-125) [Homo sapiens]
386	3	101	3	305	g1655596	1.00E-22	ribosomal protein L31 [Homo sapiens]
386	3	101	3	305	g57115	1.00E-22	ribosomal protein L31 (AA 1-125) [Rattus norvegicus]
387	3	259	3	779	g2331301	1.00E-122	ribosomal protein S4 type I [Zea mays]
387	3	259	3	779	g2345154	1.00E-120	ribosomal protein S4 [Zea mays]
387	3	259	3	779	g7546687	1.00E-116	ribosomal protein S4 [Arabidopsis thaliana]
388	2	184	2	553	g2668748	1.00E-95	ribosomal protein L17 [Zea mays]
388	2	184	2	553	g19104	8.00E-85	ribosomal protein L17-2 [Hordeum vulgare]
388	2	184	2	553	g19102	1.00E-82	ribosomal protein L17-1 [Hordeum vulgare]
389	2	152	2	457	g338447	5.00E-28	RPS16 [Homo sapiens]
389	2	152	2	457	g57714	5.00E-28	ribosomal protein S16 (AA 1-146) [Rattus rattus]
389	2	152	2	457	g200796	2.00E-27	16S ribosomal protein [Mus musculus]
390	3	158	3	476	g643074	4.00E-76	putative 40S ribosomal protein s12 [Fragaria x ananassa]
390	3	158	3	476	g6716785	1.00E-75	40s ribosomal protein S23 [Euphorbia esula]
390	3	158	3	476	g7413571	6.00E-75	putative protein [Arabidopsis thaliana]
391	1	94	34	315			
392	3	83	303	551	g57121	3.00E-18	ribosomal protein L37 [Rattus norvegicus]
392	3	83	303	551	g292441	3.00E-18	ribosomal protein L37 [Homo sapiens]
392	3	83	303	551	g1839334	3.00E-18	ribosomal protein L37 (C2-C2 zinc-finger-like) [human, HeLa cells, Peptide, 97 aa] [Homo sapiens]
393	2	174	2	523	g10433651	3.00E-80	unnamed protein product [Homo sapiens]
393	2	174	2	523	g10434617	3.00E-80	unnamed protein product [Homo sapiens]
393	2	174	2	523	g545998	6.00E-79	tricarboxylate carrier [Rattus sp.]
394	3	183	3	551			
395	1	399	1	1197	g11907599	0	protein kinase HIPK2 [Homo sapiens]
395	1	399	1	1197	g5815141	0	nuclear body associated kinase 1b [Mus musculus]
395	1	399	1	1197	g5815139	0	nuclear body associated kinase 1a [Mus musculus]
396	1	301	109	1011	g7688667	1.00E-161	PC326 protein [Homo sapiens]
396	1	301	109	1011	g2734854	1.00E-08	Mus musculus Dentin Matrix Protein 1 []
396	1	301	109	1011	g6137020	1.00E-08	dentin matrix protein-1 [Mus musculus]

397	2	105	2	316	g178281	1.00E-47	AHNAK nucleoprotein [Homo sapiens]
397	2	105	2	316	g50675	2.00E-47	desmoyokin [Mus musculus]
397	2	105	2	316	g897824	5.00E-47	AHNAK gene product [Homo sapiens]
398	1	153	202	660	g183233	1.00E-34	beta-glucuronidase precursor (EC 3.2.1.31) [Homo sapiens]
398	1	153	202	660	g3549609	2.00E-33	beta-glucuronidase [Cercopithecus aethiops]
398	1	153	202	660	g4102553	3.00E-29	mutant beta-glucuronidase [Felis catus]
399	1	161	106	588	g7022046	1.00E-36	unnamed protein product [Homo sapiens]
399	1	161	106	588	g7670456	5.00E-34	unnamed protein product [Mus musculus]
399	1	161	106	588	g8671586	1.00E-29	ataxin 2-binding protein [Homo sapiens]
400	1	153	205	663	g183233	1.00E-34	beta-glucuronidase precursor (EC 3.2.1.31) [Homo sapiens]
400	1	153	205	663	g3549609	2.00E-33	beta-glucuronidase [Cercopithecus aethiops]
400	1	153	205	663	g4102553	3.00E-29	mutant beta-glucuronidase [Felis catus]
401	3	135	651	1055	g414797	9.00E-58	pyruvate dehydrogenase phosphatase [Bos taurus]
401	3	135	651	1055	g3298607	3.00E-56	pyruvate dehydrogenase phosphatase isoenzyme 1 [Rattus norvegicus]
401	3	135	651	1055	g7688679	3.00E-53	pyruvate dehydrogenase [Homo sapiens]
402	3	129	30	416			
403	1	299	1	897	g440878	1.00E-149	onconeural ventral antigen-1 [Homo sapiens]
403	1	299	1	897	g7025507	1.00E-137	ventral neuron-specific protein 1 NOVA1 [Mus musculus]
403	1	299	1	897	g2673961	9.00E-99	astrocytic NOVA-like RNA-binding protein [Homo sapiens]
404	1	142	1	426	g4105111	1.00E-43	dehydrin 6 [Hordeum vulgare]
404	1	142	1	426	g6017938	4.00E-43	dehydrin; DHN6 [Hordeum vulgare]
404	1	142	1	426	g5738195	1.00E-28	abscisic acid response protein [Prunus dulcis]
405	2	168	2	505	g453189	9.00E-59	acyl carrier protein [Zea mays]
405	2	168	2	505	g166971	4.00E-49	acyl carrier protein III [Hordeum vulgare]
405	2	168	2	505	g166969	6.00E-41	acyl carrier protein II [Hordeum vulgare]
406	2	117	2	352	g203923	1.00E-40	diazepam binding inhibitor [Rattus norvegicus]
406	2	117	2	352	g1228089	1.00E-40	multifunctional acyl-CoA-binding protein [Rattus norvegicus]
406	2	117	2	352	g203925	1.00E-40	diazepam binding inhibitor [Rattus norvegicus]
407	3	804	3	2414	g10953883	0	ubiquitin E3 ligase SMURF2 [Homo sapiens]
407	3	804	3	2414	g10047327	0	KIAA1625 protein [Homo sapiens]
407	3	804	3	2414	g6446606	0	E3 ubiquitin ligase SMURF1 [Homo sapiens]
408	1	220	244	903	g9622856	9.00E-24	sorting nexin 15A [Homo sapiens]
408	1	220	244	903	g2529709	1.00E-23	unknown [Homo sapiens]
408	1	220	244	903	g9622854	1.00E-23	sorting nexin 15 [Homo sapiens]
							dJ20B11.1 (ortholog of rat RSEC5 (mammalian exocyst complex subunit))
409	2	168	80	583	g5823961	2.00E-87	[Homo sapiens]

409	2	168	80	583	g2827158	2.00E-84	rsec5 [Rattus norvegicus]
409	2	168	80	583	g7295804	8.00E-29	CG8843 gene product [Drosophila melanogaster]
410	2	108	194	517	g9963839	1.00E-50	lipase [Homo sapiens]
411	1	314	277	1218	g3243240	4.00E-56	syntaxin 11 [Homo sapiens]
411	1	314	277	1218	g4104685	1.00E-53	syntaxin 11 [Homo sapiens]
411	1	314	277	1218	g3248918	8.00E-46	syntaxin 11 [Homo sapiens]
412	2	143	212	640	g4512103	3.00E-57	rab11 binding protein [Bos taurus]
412	2	143	212	640	g6049150	8.00E-43	WD-containing protein [Rattus norvegicus]
413	1	122	1	366			
414	2	86	623	880			
415	3	213	183	821			
416	1	263	40	828	g9558701	3.00E-31	gag [Homo sapiens]
416	1	263	40	828	g5802824	3.00E-31	Gag-Pro-Pol protein [Homo sapiens]
416	1	263	40	828	g5802821	3.00E-31	Gag-Pro-Pol protein [Homo sapiens]
417	1	175	940	1464	g246483	1.00E-63	prohibitin [human, Peptide, 272 aa] [Homo sapiens]
417	1	175	940	1464	g206384	2.00E-63	prohibitin [Rattus norvegicus]
417	1	175	940	1464	g541732	2.00E-63	prohibitin or B-cell receptor associated protein (BAP) 32 [Mus musculus]
418	2	272	167	982	g505033	6.00E-75	mitogen inducible gene mig-2 [Homo sapiens]
418	2	272	167	982	g10727293	5.00E-33	CG14991 gene product [alt 2] [Drosophila melanogaster]
418	2	272	167	982	g7292434	5.00E-33	CG14991 gene product [alt 1] [Drosophila melanogaster]
419	1	167	16	516	g2587027	3.00E-34	HERV-E envelope glycoprotein [Homo sapiens]
419	1	167	16	516	g2587024	3.00E-34	HERV-E envelope glycoprotein [Homo sapiens]
419	1	167	16	516	g1049232	2.00E-31	HERV-E envelope protein [Human endogenous retrovirus]
420	2	59	227	403			
421	1	216	1	648	g10504238	1.00E-101	hepatocellular carcinoma-related putative tumor suppressor [Homo sapiens]
421	1	216	1	648	g7020759	7.00E-75	unnamed protein product [Homo sapiens]
421	1	216	1	648			contains similarity to Pfam domain: PF01585 (G-patch domain), Score=67.0, E
422	1	162	1	486	g3880143	1.00E-28	value=1.3e-16, N=1 [Caenorhabditis elegans]
422	1	162	1	486	g4982485	7.00E-55	apoptosis related protein APR-3 [Homo sapiens]
422	1	162	1	486	g4689122	3.00E-49	HSPC013 [Homo sapiens]

Table 7

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25:3389-3402.	ESTs: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. USA 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183:63-98; and Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value=1.06E-6 Assembled ESTs: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less Full Length sequences: fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S. and J.G. Henikoff (1991) Nucleic Acids Res. 19:6565-6572; Henikoff, J.G. and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Atwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37:417-424.	Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol. 235:1501-1531; Sonnhammer, E.L.L. et al. (1998) Nucleic Acids Res. 26:320-322; Durbin, R. et al. (1998) Our World View, in a Nutshell, Cambridge Univ. Press, pp. 1-350.	PFAM hits: Probability value= 1.0E-3 or less Signal peptide hits: Score= 0 or greater

Table 7 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, M. et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221.	Normalized quality score \geq GCG-specified "HIGH" value for that particular Prosite motif. Generally, score=1.4-2.1.
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M.S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies.	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12:431-439.	Score=3.5 or greater
TMAP	A program that uses weight matrices to delineate transmembrane segments on protein sequences and determine orientation.	Persson, B. and P. Argos (1994) J. Mol. Biol. 237:182-192; Persson, B. and P. Argos (1996) Protein Sci. 5:363-371.	
TMHMMER	A program that uses a hidden Markov model (HMM) to delineate transmembrane segments on protein sequences and determine orientation.	Sonnhammer, E.L. et al. (1998) Proc. Sixth Intl. Conf. on Intelligent Systems for Mol. Biol., Glasgow et al., eds., The Am. Assoc. for Artificial Intelligence Press, Menlo Park, CA, pp. 175-182.	
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a polynucleotide sequence selected from the group
5 consisting of:
 - a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211,
 - b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211,
 - c) a polynucleotide sequence complementary to a),
 - 10 d) a polynucleotide sequence complementary to b), and
 - e) an RNA equivalent of a) through d).
2. An isolated polynucleotide of claim 1, comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:1-211.
15
3. An isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide of claim 1.
4. A composition for the detection of expression of diagnostic and therapeutic polynucleotides
20 comprising at least one of the polynucleotides of claim 1 and a detectable label.
5. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 1, the method comprising:
 - a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction
25 amplification, and
 - b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.
6. A method for detecting a target polynucleotide in a sample, said target polynucleotide
30 comprising a sequence of a polynucleotide of claim 1, the method comprising:
 - a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and

b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof.

7. A method of claim 5, wherein the probe comprises at least 30 contiguous nucleotides.

5

8. A method of claim 5, wherein the probe comprises at least 60 contiguous nucleotides.

9. A recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide of claim 1.

10

10. A cell transformed with a recombinant polynucleotide of claim 9.

11. A transgenic organism comprising a recombinant polynucleotide of claim 9.

15

12. A method for producing a diagnostic and therapeutic polypeptide, the method comprising:

- a) culturing a cell under conditions suitable for expression of the diagnostic and therapeutic polypeptide, wherein said cell is transformed with a recombinant polynucleotide of claim 9, and
- b) recovering the diagnostic and therapeutic polypeptide so expressed.

20

13. A purified diagnostic and therapeutic polypeptide (DITHP) encoded by at least one of the polynucleotides of claim 2.

14. An isolated antibody which specifically binds to a diagnostic and therapeutic polypeptide of claim 13.

25

15. A method of identifying a test compound which specifically binds to the diagnostic and therapeutic polypeptide of claim 13, the method comprising the steps of:

- a) providing a test compound;
- b) combining the diagnostic and therapeutic polypeptide with the test compound for a sufficient time and under suitable conditions for binding; and
- c) detecting binding of the diagnostic and therapeutic polypeptide to the test compound, thereby identifying the test compound which specifically binds the diagnostic and therapeutic polypeptide.

30

16. A microarray wherein at least one element of the microarray is a polynucleotide of claim 3.

17. A method for generating a transcript image of a sample which contains polynucleotides,
5 the method comprising the steps of:
a) labeling the polynucleotides of the sample,
b) contacting the elements of the microarray of claim 16 with the labeled polynucleotides of the
sample under conditions suitable for the formation of a hybridization complex, and
c) quantifying the expression of the polynucleotides in the sample.

10

18. A method for screening a compound for effectiveness in altering expression of a target
polynucleotide, wherein said target polynucleotide comprises a polynucleotide sequence of claim 1, the
method comprising:

a) exposing a sample comprising the target polynucleotide to a compound, under conditions
15 suitable for the expression of the target polynucleotide,
b) detecting altered expression of the target polynucleotide, and
c) comparing the expression of the target polynucleotide in the presence of varying amounts of
the compound and in the absence of the compound.

20

19. A method for assessing toxicity of a test compound, said method comprising:

a) treating a biological sample containing nucleic acids with the test compound;
b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at
least 20 contiguous nucleotides of a polynucleotide of claim 1 under conditions whereby a specific
hybridization complex is formed between said probe and a target polynucleotide in the biological
25 sample, said target polynucleotide comprising a polynucleotide sequence of a polynucleotide of claim 1
or fragment thereof;
c) quantifying the amount of hybridization complex; and
d) comparing the amount of hybridization complex in the treated biological sample with the
amount of hybridization complex in an untreated biological sample, wherein a difference in the amount
30 of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

20. An array comprising different nucleotide molecules affixed in distinct physical locations on
a solid substrate, wherein at least one of said nucleotide molecules comprises a first oligonucleotide or
polynucleotide sequence specifically hybridizable with at least 30 contiguous nucleotides of a target

polynucleotide, said target polynucleotide having a sequence of claim 1.

21. An array of claim 20, wherein said first oligonucleotide or polynucleotide sequence is completely complementary to at least 30 contiguous nucleotides of said target polynucleotide.

5

22. An array of claim 20, wherein said first oligonucleotide or polynucleotide sequence is completely complementary to at least 60 contiguous nucleotides of said target polynucleotide

23. An array of claim 20, which is a microarray.

10

24. An array of claim 20, further comprising said target polynucleotide hybridized to said first oligonucleotide or polynucleotide.

25. An array of claim 20, wherein a linker joins at least one of said nucleotide molecules to said solid substrate.

15

26. An array of claim 20, wherein each distinct physical location on the substrate contains multiple nucleotide molecules having the same sequence, and each distinct physical location on the substrate contains nucleotide molecules having a sequence which differs from the sequence of nucleotide molecules at another physical location on the substrate.

20

27. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

- a) an amino acid sequence selected from the group consisting of SEQ ID NO:212-422,
- 25 b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:212-422,
- c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:212-422, and
- d) an immunogenic fragment of an amino acid sequence selected from the group consisting
- 30 of SEQ ID NO:212-422.

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